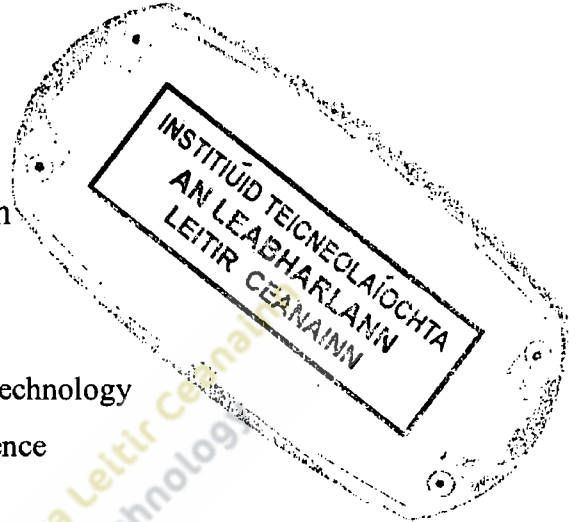
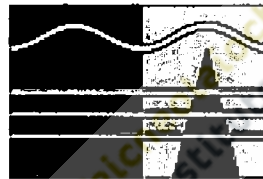


# Factors affecting the concentration of domoic acid in scallop, *Pecten maximus*

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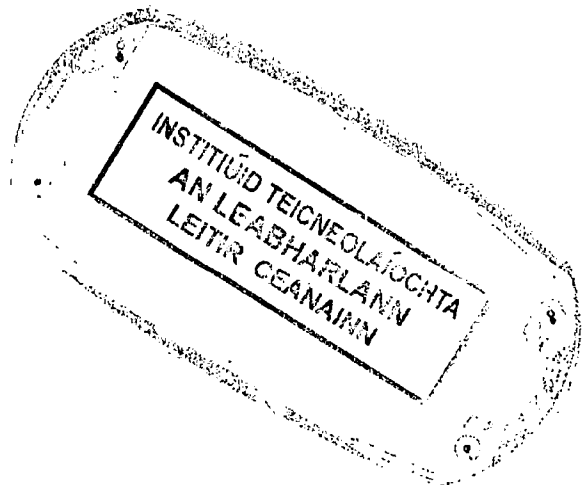
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## List of Papers

### Papers published:

- Paper 1      Bogan, Y.M., A.L. Harkin, J. Gillespie, D.J. Kennedy, P. Hess, J.W. Slater. 2006. The influence of size on domoic acid concentration in king scallop, *Pecten maximus* (L.). Harmful Algae (in press).
- Paper 2      Bogan, Y.M., K. Bender, A. Hervas, D.J. Kennedy, J.W. Slater, P. Hess. 2006. Spatial variability of domoic acid concentration in king scallops *Pecten maximus* off the southeast coast of Ireland. Harmful Algae (in press).
- Paper 3      Bogan Y.M., D.J. Kennedy, A.L. Harkin, J. Gillespie, P. Hess, J.W. Slater. 2006. Comparison of domoic acid concentration in king scallops, *Pecten maximus* from seabed and suspended culture systems. J. Shellfish Res. 25 (1), 129-135.
- Paper 4      Bogan, Y.M., D. J. Kennedy, A.L. Harkin, J. Gillespie, B.J. Vause, B.D. Beukers-Stewart, P. Hess, J.W. Slater. 2006. Variation in domoic acid concentration in king scallop (*Pecten maximus*) from fishing grounds around the Isle of Man. Harmful Algae (in press).

### Papers in preparation:

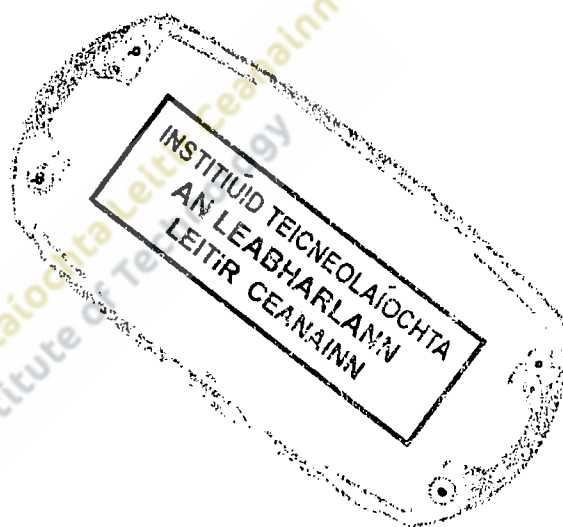
- Paper 5      Slater J.W., Y.M. Bogan, C. Greenwood, A.L. Harkin, D. Kennedy, J. Gillespie, P. Hess. 2006. Does domoic acid concentration vary with king scallop (*Pecten maximus*) size?

- Paper 6      Bogan, Y.M., A. Hervas, J.W. Slater, P. Hess. 2006. Confirming spatial variability of domoic acid concentration in king scallops *Pecten maximus* off the southeast coast of Ireland.
- Paper 7      Bogan, Y.M., D. J. Kennedy, J. Gillespie, P. Hess, J.W. Slater. 2006. An investigation of the uptake of non-cellular domoic acid by king scallop, *Pecten maximus*.
- Paper 8      Bogan, Y.M., P. McCarron, J. Kilcoyne, J. Hickey, A. Hervas, P. Hess, J.W. Slater. 2006. Detection of domoic acid in the king scallop *Pecten maximus* off the southeast coast of Ireland using Jellett Rapid® Test Kit for ASP in parallel with the HPLC regulatory method.



## Glossary

ANOVA – Analysis of Variance  
AOAC – Association of Analytical Chemists  
ASP – Amnesic Shellfish Poisoning  
AZP – Azaspiracid Shellfish Poisoning  
BOHAB – Biological Oceanography of Harmful Algal Blooms  
CE – Capillary Electrophoresis  
CV – Co-efficient of Variation  
DA – Domoic Acid  
DAP – Domoic Acid Poisoning  
DSP – Diarrhetic Shellfish Poisoning  
ELISA – Enzyme Linked ImmunoSorbent Assay  
EU – European Union  
FSA – Food Safety Authority (UK)  
FSAI – Food Safety Authority of Ireland  
HAB – Harmful Algal Bloom  
HAE – Harmful Algal Event  
HPLC – High Performance Liquid Chromatography  
ICES – International Council for the Exploration of the Sea  
IOM – Isle of Man  
LC-FLD – Liquid Chromatography - Fluorescence Detection  
LC-MS – Liquid Chromatography - Mass Spectrometry  
LC-UV – Liquid Chromatography – Ultra Violet Detection  
LOD – Limit of Detection  
LOQ – Limit of Quantification  
MI – Marine Institute  
NSP – Neurotoxic Shellfish Poisoning  
PCR – Polymerase Chain Reaction  
PDA - Photo-Diode Array Detection  
PSP – Paralytic Shellfish Poisoning



RGH – Relative Gonad Height

RTDI – Research, Technology, Innovation and Development

SAX – Strong Anionic Exchange

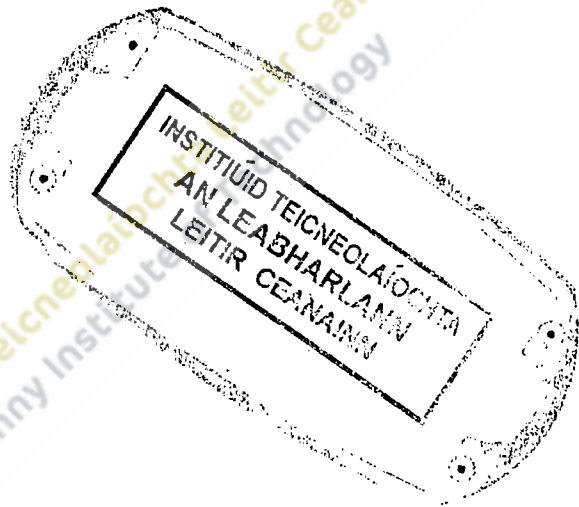
SPATT – Solid Phase Adsorption Toxin Tracking bags

SPE – Solid Phase Extraction

STX – Saxitoxin

TLC – Thin Layer Chromatography

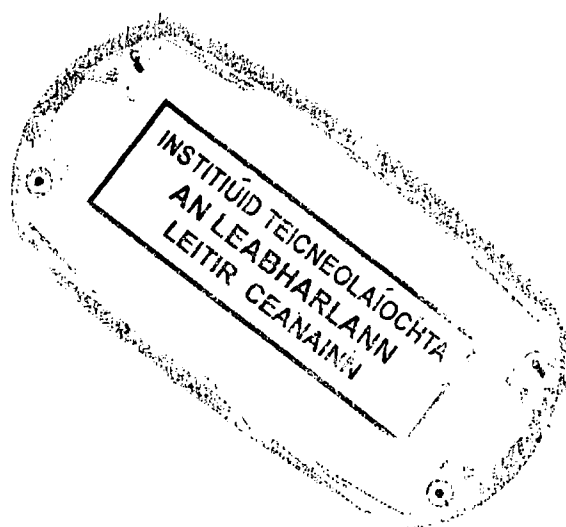
UV – Ultra Violet Detection



## Abstract

Domoic acid, a neurotoxin produced by some *Pseudo-nitzschia* species, can accumulate in shellfish, consumption of which can result in Amnesic Shellfish Poisoning. Since its detection in Irish king scallop, *Pecten maximus*, regulatory monitoring of toxin levels in product entering the human food chain has been undertaken. Only limited data exist on factors that may influence variability in scallop DA concentration in the field.

DA concentration in scallop tissues from a range of sites around Ireland, analysed using HPLC-PDA, exhibited high concentrations in hepatopancreas (max. 3834.4  $\mu\text{g.g}^{-1}$ ), much lower in gonad (max. 61.3  $\mu\text{g.g}^{-1}$ ) and even lower concentrations in adductor muscle (max. 31.8  $\mu\text{g.g}^{-1}$ ). Toxin concentration in hepatopancreas and scallop size usually exhibited no relationship and there was little support for the hypothesis that shellfish size influenced toxin concentration. DA concentration exhibited site-specific relationships with water depth. Toxin concentration in suspended scallops compared to seabed scallops exhibited a statistically significant difference on only one sampling occasion. Attempts to correlate the occurrence of DA in bivalves with the abundance of cells of *Pseudo-nitzschia* species were unsuccessful. Given the extent of field variability in DA concentration, a landings-based approach to toxin management rather than an area-based approach would reduce the risks of poisoning.



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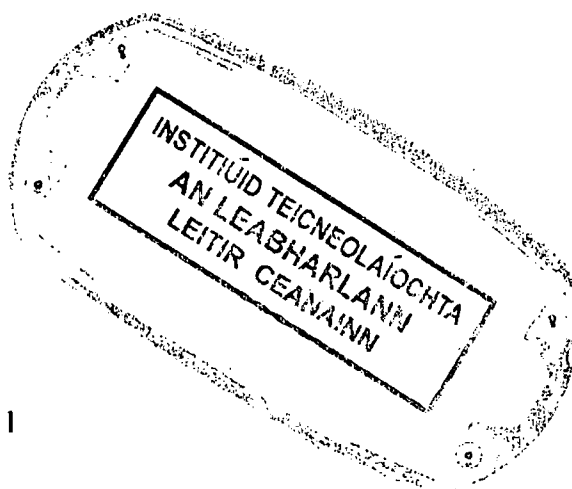
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## 1. General Introduction

### 1.1. Phytoplankton and harmful algal blooms

Marine phytoplankton are microscopic algae, often single-celled, that inhabit the photic zones of the oceans. They range in size from 2  $\mu\text{m}$  to 2 mm, with most of the cells reaching 10-50  $\mu\text{m}$  (cf. Horner 2002). The main classes are the Bacillariophyceae (diatoms), Dinophyceae (dinoflagellates), Raphidophyceae (raphidophytes), Prymnesiophyceae (prymnesiophytes, haptophytes), Dictyochophyceae (silicoflagellates) and Cyanophyceae (cyanobacteria). There are conservatively estimated to be about 500 genera and 4000 species of phytoplankton (Sournia et al. 1991). As the primary producers supporting the marine food web they are the basis of all animal production in the sea. They play an essential role in the global biogeochemical cycle by producing about a quarter of the world's oxygen and through their utilisation of carbon in photosynthesis.

Marine phytoplankton are a primary food source for filter-feeding bivalve shellfish as well as larvae of commercially important crustaceans, hence proliferation of planktonic algae is in most cases beneficial for fisheries and aquaculture. However, when forming a harmful algal bloom (HAB) or harmful algal event (HAE), as they are sometimes referred, toxic and non-toxic microalgae may have a negative effect on the environment, economy and human health (Daranas et al. 2001). The term "harmful algal bloom" is used to describe the often visible blooms of algae that can kill fish, make shellfish poisonous and cause numerous other effects on wildlife, humans and economy (Hoagland et al. 2002). The threats posed by the increasing frequency, duration and distribution of harmful algal blooms (HAB's) are now recognised as a global concern (Anderson 1989, Smayda 1990, Hallegraeff 1993, Anderson et al. 2002). The global expansion of HAB's has been linked to eutrophication as a result of nutrient overloading from sewage, atmospheric deposition, groundwater flow, as well as agricultural and aquaculture runoff and discharge (Anderson et al. 2002). HAB's result from the unusual growth of phytoplankton and impact on the environment using different mechanisms; some bloom

so densely that they generate anoxic conditions killing marine life forms, others impact by physically clogging the gills of fish and other organisms, and yet others produce phycotoxins capable of causing death of marine mammals, seabirds, mass mortalities of fish, human illness and even death.

Of the five thousand species of phytoplankton described (Sournia et al. 1991) some ninety-seven species are known to produce toxins (Moestrup 2004). These species can enter the food chain as a result of the consumption of contaminated shellfish, for example bivalves which have been filter feeding on the toxic phytoplankton, proving harmful or even fatal to humans.

The risks to public health posed by HAB's have led to increased efforts in Ireland and other countries to understand this global phenomenon. Several different hypotheses have been proposed to explain the increased incidence of HAB's including amongst them an improved understanding of algal toxins by scientists and the inherent increase in monitoring performed nowadays. Considerable evidence exists for an association between eutrophication, resulting from anthropogenic inputs from industrial and domestic waste and agricultural run-off, and the formation of algal blooms (Anderson et al. 2002). Early evidence for this relationship was provided by the increased frequency of algal blooms in Tolo Harbour in Hong Kong between 1976-86 and the increases in population that had escalated the amount of nutrients, mainly from untreated urban waste, discharged to the Harbour (Lam & Ho 1989). Similar reports of a relationship with increased nutrient loading have been reported from many other locations and have led to concerted efforts throughout the world to reduce nutrient discharges. Changing weather patterns and increases in sea temperatures attributed to global warming have also been proposed as having a role in the expansion of harmful algal blooms.

Bivalve molluscs can act as vectors by transmitting phycotoxins that can cause intoxication to humans and animals. Five types of shellfish poisoning are known worldwide: paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP), azaspiracid shellfish poisoning (AZP) and amnesic

shellfish poisoning (ASP). With the exception of the latter, which is caused by diatoms, all the other forms of shellfish poisoning are caused by dinoflagellate species. The phytoplankton species and the algal toxins involved with each type of shellfish poisoning are provided in Table 1.

Type of poisoning	Source of toxin	Algal toxins
PSP	<i>Alexandrium spp.</i>	Saxitoxin
DSP	<i>Dinophysis spp.</i> <i>Dinophysis spp.</i>	Okadaic acid Pectenotoxin
NSP	<i>Gymnodinium breve</i>	Brevetoxin
AZP	<i>Protoperdinium crassipes</i> (possibly)	Azaspiracid
ASP	<i>Pseudo-nitzschia spp.</i>	Domoic acid

Table 1. Types of shellfish poisoning

## 1.2. Shellfish poisoning in Ireland

Four types of shellfish poisoning have been recorded in Ireland to date: paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), azaspiracid shellfish poisoning (AZP) and amnesic shellfish poisoning (ASP).

PSP toxins are potent hydrophilic neurotoxins. PSP was one of the first known shellfish poisonings and human fatalities have resulted from the consumption of contaminated shellfish. Symptoms of PSP in a mild case include facial paraesthesia, drowsiness, tingling, incoherent speech, headache, vomiting and diarrhoea. In more extreme cases, symptoms may include respiratory paralysis leading to death. The standard mouse bioassay method forms the basis of most monitoring programmes (AOAC, 2000a). Other methods used include ELISA (Garthwaite et al. 2001) and LC-FLD (Lawrence et al.



2004). A regulatory level of 80 µg STX-equivalent per 100g of tissue has been adopted by most countries for PSP toxin.

DSP toxins are lipophilic and are one of the most important toxin groups as a result of their wide distribution (Hallegraeff 1993). The main toxins that cause DSP are okadaic acid and dinophysistoxins. Gastrointestinal symptoms can occur within 30 minutes of consumption and include incapacitating diarrhoea, nausea and chills (Van Egmond et al. 1993). No human fatalities have ever been reported from this syndrome. Concerning detection methods, the EU Commission Decision 2002/225/EC states that as well as customary biological methods, chemical methods and other assays should be accepted if it is demonstrated that these methods are not less effective than the mouse bioassay (diethyl ether step) (Yasumoto et al. 1984). For example, although it is not a statutory requirement, DSP analysis can be carried out using LC MS-MS (Hess 2001). The EU regulatory limit for DSP toxins is 160 µg OA-equivalent.kg<sup>-1</sup>.

In 1995 a shellfish-poisoning incident occurred in the Netherlands in which eight people became ill after consuming blue mussels, *Mytilus edulis*, from Killary Harbour on the west coast of Ireland (Satake et al. 1997, 1998). Human symptoms of AZP poisoning were typical of DSP poisoning and included diarrhoea, stomach cramps, nausea and vomiting. The determination of azaspiracid in shellfish relies on the use of LC-MS and LC-MS-MS methods. Multi-toxin methods (Quilliam et al. 2001, McNabb et al. 2005) are currently under development, however lack of standards and reference materials for AZA's has limited the development of analytical methods. A regulatory limit of 160 µg.kg<sup>-1</sup> of azaspiracids was introduced by the European Union in 2002. The source of the AZP toxins in Ireland has been tentatively suggested as the dinoflagellate, *Protoperdinium crassipes* (James et al. 2003), but this remains unconfirmed to date.

ASP sometimes referred to as domoic acid poisoning (DAP) in humans, is caused by the consumption of shellfish intoxicated with the neurotoxin domoic acid (DA). This type of shellfish poisoning was first reported in Canada over eighteen years ago and the source of the toxin traced to a pennate diatom of the genus *Pseudo-nitzschia*.



### 1.3. Amnesic shellfish poisoning (ASP)

#### 1.3.1. Occurrence of ASP outbreaks

The first incidence of amnesic shellfish poisoning was described in 1987 from Prince Edward Island, Canada. One hundred and seven human cases of shellfish poisoning were reported following the consumption of blue mussel, *Mytilus edulis* (Quilliam and Wright 1989, Perl et al. 1990). Most of the cases experienced gastroenteritis with some people presenting symptoms of memory loss. Three elderly people died eleven to twenty-four days after the consumption of mussels. The cause of death in these three patients was septic shock and pneumonia and all exhibited severe neurological disorders (Todd 1993). General symptoms experienced by other patients included nausea, abdominal cramps, disorientation, somnolence, lethargy, seizures and a decreased level of consciousness. A short-term memory loss was observed in 25 % of the patients with the most severely affected experiencing memory loss five years after the event (Perl et al. 1990). To date no specific antidote has been developed for DA poisoning, treatment comprising only re-hydration and support. The source of the shellfish poisoning was traced to *Mytilus edulis* that had been cultivated in three river estuaries (Cardigan, Brudnell and Murray) on the east coast of Prince Edward Island. Analysis of the shellfish tissue led to isolation of the toxin and its subsequent identification as domoic acid. It was established that the mussels had been filtering a toxic phytoplankton species called *Pseudo-nitzschia multiseries* and this was identified as the source of the toxin responsible for the human poisoning. Laboratory culture of the diatom *Pseudo-nitzschia multiseries* confirmed the production of DA at levels from 1 – 20 pg.cell<sup>-1</sup> (Bates et al. 1989). DA concentrations in the mussel tissue as high as 900 µg.g<sup>-1</sup> were reported in this toxic event (Addison & Stewart 1989). Further incidences of toxic *Pseudo-nitzschia* blooms were described in Canada in each of the following three years, but as a result of an expanded monitoring programme no human intoxications resulted.

Since this initial Canadian outbreak there have been numerous reports of ASP from other parts of the world. The toxin has been reported not only in bivalve molluscs but also in

many other marine species including marine mammals, sea birds, crustaceans and planktivorous fish.

Frequent blooms of *Pseudo-nitzschia* have been reported in Monterey Bay, California in past years. In 1991 both brown pelicans, *Pelecanus occidentalis* and Brandt's cormorants, *Phalacrocorax penicillatus*, were reported dead on the beaches of Monterey Bay after consuming the filter feeding northern anchovy, *Engraulis mordax*, contaminated with DA (Work et al. 1993). Concentrations up to 485  $\mu\text{g.g}^{-1}$  DA were detected in the hepatopancreas of the anchovies. The birds displayed a characteristic slow, side-to-side head motion and also exhibited torticollis or wryneck. Upon analysis of the stomach contents of the dead and sick birds, DA was detected in the hepatopancreas and flesh of the anchovies. This toxic event was significant due to the fact that the vector for toxin transfer was not a shellfish species. In the same year DA concentrations in the edible portion of razor clams, *Siliqua patula*, in Washington reached 147  $\mu\text{g.g}^{-1}$  resulting in the closure of harvesting areas. Toxin concentrations were extremely variable from one site to the next (Wekell et al. 2002). High concentrations of DA exceeding the regulatory limit of 20  $\mu\text{g.g}^{-1}$  were also recorded in the Dungeness crab, *Cancer magister*, from both the Washington and Oregon coasts in the same year, though only in the crab viscera (Wekell et al. 1994).

DA was first confirmed in European waters in mussels, *Mytilus galloprovincialis*, harvested from Galicia, northwest Spain during 1994, although toxin concentrations remained below the regulatory limit (Miguez et al. 1996). In subsequent years both scallops and mussels from Spain became intoxicated and in scallops particularly, high DA concentrations persisted for months and even years (Arévalo et al. 1998). In 1996 over a hundred brown pelicans (*Pelecanus occidentalis*) died after feeding on DA-contaminated chub mackerel (*Scomber japonicus*) in Mexico. Live pelicans displayed symptoms of disorientation and difficulty in swimming (Sierra Beltrán et al. 1997). The first confirmed death of marine mammals due to DA poisoning occurred in Monterey Bay, California during 1998. Over 400 Californian sea lions, *Zalophus californianus*, died following the ingestion of anchovies, *Engraulis mordax*,

contaminated with DA in the viscera at concentrations in excess of  $223 \mu\text{g.g}^{-1}$  (Lefebvre et al. 1999).

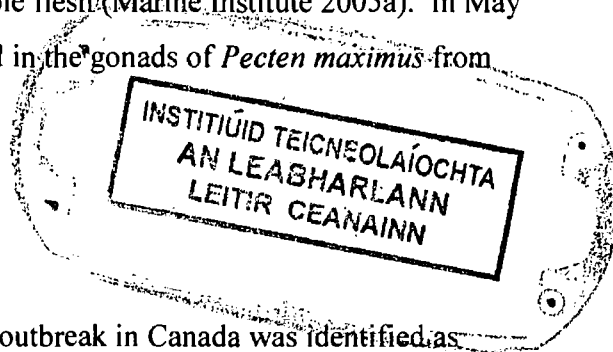
DA concentrations exceeded the regulatory limit in king scallop, *Pecten maximus*, from waters around northern and western Scotland in 1999 (Gallacher et al. 2001). High DA concentrations led to the most extensive fishery closure to date due to this toxin, covering an area of  $49,000 \text{ km}^2$  and persisting throughout much of the year 2000 (Campbell et al. 2001). In December 1999, high concentrations of DA in shellfish from Irish waters resulted in the prohibition of all fresh shellfish exports over the millennium period (McMahon & Silke 2000, James et al. 2005). In Portugal, concentrations of DA as high as  $571 \mu\text{g.g}^{-1}$  of viscera were reported in the swimming crab, *Polybius henslowii*, during 2002. This was the first report of DA in this species (Costa et al. 2003).

During 2003 forty-three dolphins along the Californian coast were stranded and subsequently died due to disorientation resulting from DA poisoning (Gollan 2003). Concentrations of DA as high as  $241 \mu\text{g.g}^{-1}$  of digestive gland were reported in the common cuttlefish, *Sepia officinalis*, from the Portuguese coast during 2004 (Costa et al. 2005).

During April and May 2005 shellfish production areas in Cork and Kerry were closed due to high levels of DA in mussels, *Mytilus edulis*. Toxin concentrations in Ardroom, Co. Cork reached  $444.9 \mu\text{g.g}^{-1}$  DA in mussel whole flesh (Marine Institute 2005a). In May 2005 DA levels of  $123.8 \mu\text{g.g}^{-1}$  were reported in the gonads of *Pecten maximus* from Valentia River (Marine Institute 2005b).

### 1.3.2. Sources of ASP toxin

The source of domoic acid in the initial ASP outbreak in Canada was identified as *Pseudo-nitzschia multiseries* (Bates et al. 1989). The high toxin concentrations in shellfish in Ardroom and Valentia River recorded during April and May 2005 coincided with a bloom of *Pseudo-nitzschia* at densities in excess of  $100,000 \text{ cells.l}^{-1}$ , the trigger



level for a *Pseudo-nitzschia* bloom above which shellfish toxicity might be anticipated (Bates et al. 1998). The *Pseudo-nitzschia* species responsible for toxin production in this recent Irish incident were tentatively identified by electron microscopy as *Pseudo-nitzschia australis* and *Pseudo-nitzschia seriata*. The genus *Pseudo-nitzschia* Peragallo comprises about 30 species (including species variants), of which at least ten have now been confirmed to produce the neurotoxin DA in the field or in culture (Bates 2000). The only DA-producing diatom that does not belong to the genus *Pseudo-nitzschia* is the benthic species *Amphora coffeaeformis* (Agardh) Kützinger (Shimizu et al. 1989, Maranda et al. 1990, Bates, 2000), although recently another benthic diatom, *Nitzschia navis-varingica*, isolated from a shrimp pond in Vietnam, has been shown to be capable of DA production (Lundholm & Moestrup 2000, Kotaki et al. 2000, 2004). Many of the potentially toxic *Pseudo-nitzschia* species are widely distributed and this may account for the widespread outbreaks of ASP in locations around the world (Hasle 2002).

### 1.3.3. *Pseudo-nitzschia* and DA production

Most investigations of DA production by *Pseudo-nitzschia* species have been performed on *P. multiseries*, the species isolated following the ASP event in Canada in 1987 and shown to produce DA in culture. The production of DA by this species in batch culture occurred only in the late exponential and stationary phases of the cell growth cycle i.e. as cell division declined and eventually ended, so DA production was enhanced (Pan et al. 1996a, 1996b, 1998, Bates et al. 1998). This inverse correlation between toxin production and cell growth rate was attributed to the response to some type of stress, such as phosphate or silicate limitation during batch culture (Pan et al. 1998). This pattern of DA production differed from that reported with other *Pseudo-nitzschia* species, for example, *P. australis* was reported to produce DA in mid-exponential phase (Garrison et al. 1992), although Cusack (2002) reported DA production by this species in both the late exponential and stationary phase depending on light intensity. By contrast, the production of DA by *P. pseudodelicatissima* was reported to be highest during the early exponential phase (Pan et al. 2001, Lundholm et al. 2003). Toxin production by *P. seriata* is even less well understood; traces being observed during the exponential growth

phase and highest amounts during the late stationary phase, though production rate varied with temperature, being greater at 4 °C than 15 °C (Lundholm et al. 1994).

Many studies have been undertaken of both physical and biological factors that might influence toxin production by *Pseudo-nitzschia* species. The addition of some nutrients and the limitation of others have both been shown to enhance DA production. For example, nitrogen has been reported to be an essential component for both the synthesis of DA and for cell growth (Bates et al. 1991). In laboratory culture, *Pseudo-nitzschia multiseries* ceased production of DA during the stationary phase of growth when nitrate was absent from the culture medium. Supplementation of the medium with nitrate resulted in a resumption in DA production (Bates et al. 1991). In the field, the results from several studies have suggested a correlation between environmental pulses of nitrate and increased abundance of *Pseudo-nitzschia* cells although to date such correlations have not been extended to include DA toxin concentration (Marchetti et al. 2004). It has been suggested that the absence of a correlation in field studies between DA concentration and the number of *Pseudo-nitzschia* cells, in contrast to the findings from laboratory cultures, may be due to the influence of complex physical and biological processes under natural conditions (Trainer et al. 1998). In contrast to nitrogen, limitation of both silicate and phosphate during the stationary phase resulted in enhanced toxin production (Bates 1991, 1998, Pan et al. 1996a, 1996b, 1996c).

A few investigations have examined the role of trace metals on the production of DA. Increased germanium (Ge) concentrations inhibited the growth of *P. multiseries* and DA production ceased in laboratory cultures, whereas by contrast it has been suggested that increased lithium (Li) concentrations might have been responsible for both sustaining and enhancing DA production in the field (Zhiming & Subba Rao 1998, Subba Rao et al. 1998). Laboratory studies also demonstrated enhanced DA production in the presence of added iron (Fe) (Bates et al. 2001). Evidence exists that DA may have a chelating role in the presence of low concentrations of iron and copper and it was suggested that increased DA production may be associated with increasing the availability of these metals under conditions of iron or copper limitation (Rue & Bruland 2001, Maldonado et al. 2002).

Blooms of toxic *Pseudo-nitzschia* species may also be related to physical factors in the environment as well as chemical factors. *Pseudo-nitzschia* species were capable of growth over a broad temperature range between 5 °C and 25 °C (Seguel 1991). At lower temperatures, growth of *Pseudo-nitzschia multiseries* and the production of DA were demonstrated between 13 °C to as low as 0 °C (Smith et al. 1993). Field observations suggest that favourable winds that may be responsible for pulses of nutrients may maximise blooms of toxic *Pseudo-nitzschia* species. High rainfall following an exceptionally dry summer was suggested as a contributory factor for the phytoplankton blooms associated with the Canadian ASP outbreak of 1987 and the toxic bloom in Washington of 1991 (Smith et al. 1990, Horner & Postel 1993).

The relationship between the presence of bacteria and DA production during batch culture of *Pseudo-nitzschia* species has also been investigated. DA production by *P. multiseries* was enhanced by the presence of several bacterial species although bacteria were not essential for DA production (Bates et al. 1995, 2004). Variability in DA production, even with the same *Pseudo-nitzschia* species, has been reported upon the introduction of bacteria into cultures (Kaczmarek et al. 2005).

#### 1.3.4. Accumulation and depuration of DA in bivalves

The rate of accumulation and depuration of DA in bivalves has been shown to be species-specific and have a wide-ranging variability.

For example, the bay scallop, *Argopecten irradians*, accumulated concentrations of DA up to 60 µg.g<sup>-1</sup> in the hepatopancreas within 84 hrs after feeding on toxic *P. multiseries*. Concentrations decreased to 5 µg.g<sup>-1</sup> after 48 hrs of depuration (Douglas et al. 1997). Sea scallop, *Placopecten magellanicus*, incorporated DA within 24 hrs of exposure to toxic *P. multiseries* cells, with increased uptake after 6 days exposure. DA reached a maximum concentration of 3108 µg.g<sup>-1</sup> in the hepatopancreas with only trace levels in the adductor muscle. Toxin concentrations in the hepatopancreas after 14 days exposure to a non-toxic diet remained high at 752 µg.g<sup>-1</sup> in the hepatopancreas (Douglas et al. 1997).



In field studies with king scallop, *Pecten maximus*, DA toxin accumulated in the hepatopancreas to high concentrations, to much lower concentrations in the mantle, even lower concentrations in the gonad and generally only trace levels in the adductor muscle (Arévalo et al. 1998, Campbell et al. 2001, Blanco et al. 2002). DA concentrations in the king scallop hepatopancreas exceeding  $2000 \mu\text{g.g}^{-1}$  have been reported (Arévalo et al. 1998, James et al. 2005). By contrast, investigations of DA concentration in queen scallop, *Aequipecten opercularis*, demonstrated in field studies that the toxin is not accumulated by this species (Shammon et al. 2003).

With the Pacific oyster, *Crassostrea gigas* and Californian mussel, *Mytilus californianus*, maximum DA concentration occurred after only four hours exposure to toxic *Pseudo-nitzschia* cells (Jones et al. 1995).

Although the accumulation of DA in bivalves may occur rapidly over several days, the elimination of the toxin is a much slower process and can require months or even years. Slow depuration of DA toxin from king scallop has been widely reported (Arévalo et al. 1997, Blanco et al. 2002) and was responsible for prolonged closures in Scottish scallop fisheries (Gallacher et al. 2001, Smith et al. 2005). In a laboratory study of the DA depuration rate of toxic king scallop over a 295-day period, it was estimated that 416 days would be required for scallops intoxicated with DA to reach levels that would allow the scallops to be deemed safe for human consumption (Blanco et al. 2002a). After a nineteen-month depuration trial with sea scallop, *Placopecten magellanicus*, the DA concentration in the hepatopancreas was only slightly lower than at the beginning of the depuration period (Stewart et al. 1998). The razor clam, *Siliqua patula*, has a toxin retention time estimated to be more than two years, equal to or even slower than that in *Pecten maximus* (Horner et al. 1993, Drum et al. 1993, Wekell et al. 1994, Shumway et al. 1995). It has been suggested that razor clams contain both high and low affinity receptor sites for kainic acid and may contain more than one subtype of glutamate receptor, thereby allowing the clams to function normally in a marine environment that often contains high concentrations of DA (Trainer & Bill 2004). Similar slow depuration

of DA from the horse mussel, *Modiolus modiolus*, has been reported in field studies (Stewart et al. 1998).

By contrast, DA has been shown to depurate at a much faster rate in the mytilids, *Mytilus edulis* (Wohlgelassen et al. 1992), *M. californianus* (Whyte et al. 1995) and *M. galloprovincialis* (Blanco et al. 2002b). In a laboratory study blue mussels, *Mytilus edulis*, depurated more than 50 % of DA within twenty-four hours (Novaczek et al. 1992). Detoxification in this bivalve appears to be dependent on environmental conditions and biological factors. According to Novaczek et al. (1992) the rate of DA depuration by the blue mussel was increased in warmer water, though the converse has been reported in the mussel, *Mytilus galloprovincialis* (Blanco et al. 2002b). The depuration rate was also reduced in response to lowered salinity in this latter species (Blanco et al. 2002b). Rapid rates of DA depuration have also been reported for contaminated New Zealand green-lipped mussels, *Perna canaliculus*, (Mackenzie et al. 1993) and other bivalve species such as *Mya arenaria* (Gilgan et al. 1990) and *Callista chione* (Fernández et al. 2000).

Differences in the rate of depuration between bivalve species have been attributed to different mechanisms used by shellfish in dealing with DA. Stewart et al. (1998) demonstrated that *Mytilus edulis* and *Mya arenaria*, both species that depurate rapidly, had different bacterial gut microflora to scallops, species that generally depurate more slowly. It was suggested that the slow rate of depuration in scallop might be due to the toxin being sequestered in the hepatopancreas of these slow-depurating species and thus largely unavailable for bacterial utilisation (Stewart et al. 1998).

To alleviate the impact of financial losses on the shellfish industry, especially the scallop fishery, several studies have been performed to investigate potential detoxification procedures. Scallops contaminated with high DA concentrations when transferred to sites free of toxic phytoplankton, with the intention of reducing toxin intake and accelerating the rate of depuration by flushing with non-toxic algae, showed only limited change in the DA concentration (McKenzie and Bavington 2002). Chlorine treatments,

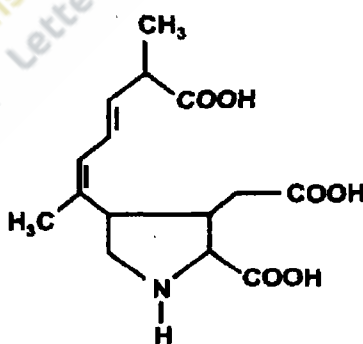


ozone treatments, temperature and salinity have all been investigated in trials to develop methods to eliminate toxins from water and bivalves with limited success to date (Novaczek et al. 1992, Rositano et al. 1998, Blanco et al. 2002b, Nicholson et al. 2003).

## 1.4. Domoic acid (DA): chemistry, stability and detection

### 1.4.1. Chemistry and pharmacology

Following the Prince Edward Island outbreak of ASP in mussels, the neurotoxin DA was isolated and identified within five days as the causative agent of the toxicity. This was not the first time that this compound had been identified, it having previously been isolated and characterised from the red algae *Chondria armata* in 1958 (Takemoto and Daigo 1958) and used in low doses in Japan as an ascaricidal agent. DA is a naturally occurring, hydrophilic algal toxin belonging to the kainoid class of compounds and displays properties typical of a neuroexcitatory amino acid. It has the molecular formula  $C_{15}H_{21}NO_6$  and a molecular weight of 311.14 Daltons. The chemical structure of this tricarboxylic acid with a secondary amino group is shown in Figure 1.



**Figure 1.** Structure of the DA molecule.

This toxin is particularly insidious as it is not destroyed by heating, although the toxicity of shellfish tissue can decrease during boiling as some of the toxin can be transferred to the surrounding liquid (Leira et al. 1998, McCarron and Hess 2006).

Ten isomers of DA have been identified; iso-domoic, the isomers A, B, C, D, E, F, G, H and epi-domoic, the diastereomer at the 5' position, though these isomers are not always present in shellfish tissues containing ASP toxin (Figure 2). The isomers are less toxic than DA (Hampson et al. 1992). Iso-domoic and both isomers G and H have been isolated from red algae *Chondria armata* (Zaman et al. 1997). In nature DA is much more abundant than the iso-domoic acids.

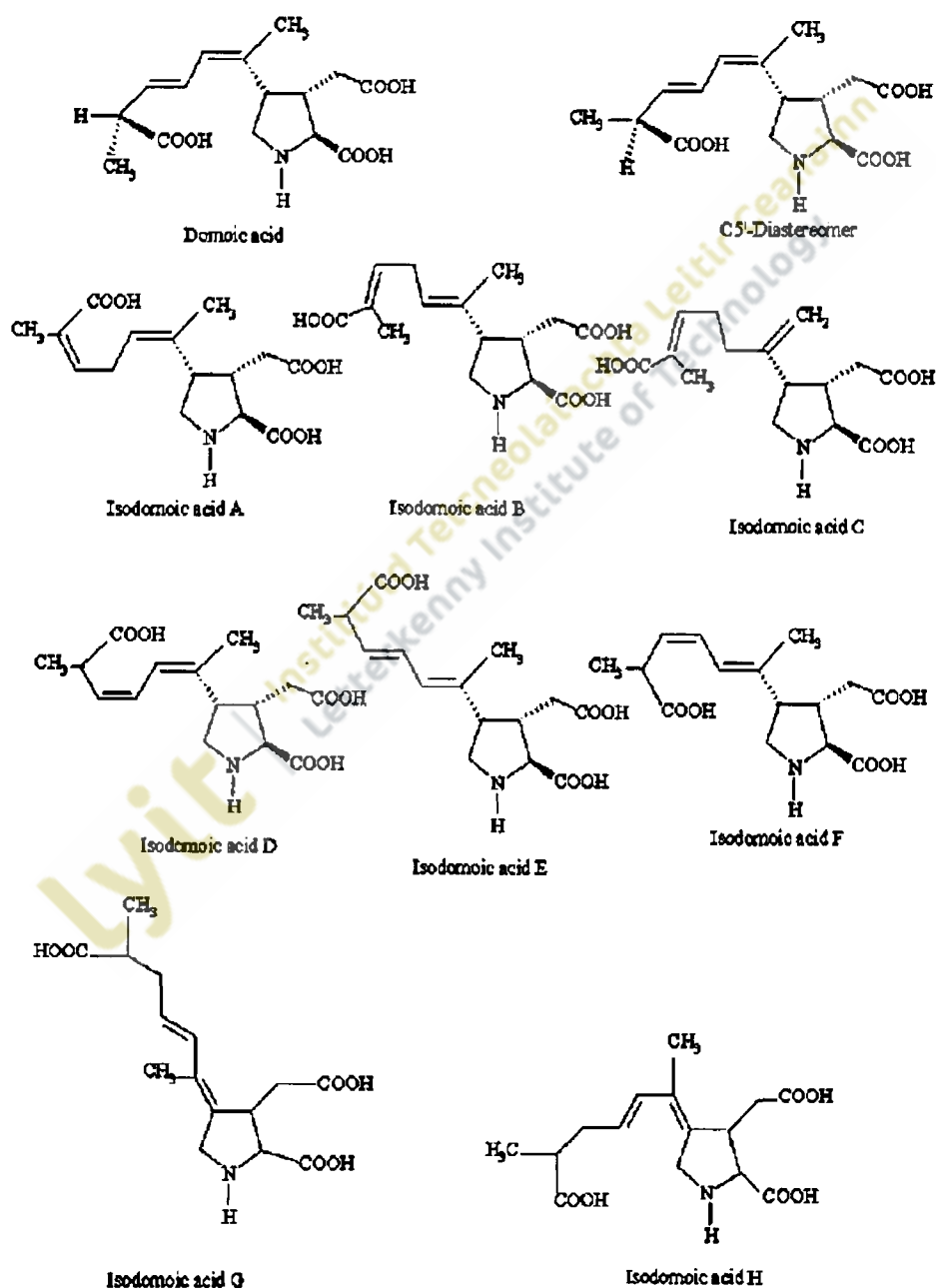


Figure 2. Isomers of domoic acid.

The pharmacological properties of DA resemble those of its analogue kainic acid, though DA is three times more potent. Like kainic acid, DA belongs to a group of excitatory neurotransmitters that bind irreversibly to specific glutamate receptors in neuronal cells (Debonnel et al. 1989). This leads to depolarisation of the cells causing them to transmit impulses continuously and eventually results in apoptosis (Wright 1995, Skifter et al. 2000). Comparative pharmacological studies indicate that the DA isomers bind less strongly to the glutamate receptor proteins than DA itself, suggesting that they are not as toxic as the parent amino acid. This toxin is a powerful convulsant causing excitotoxic degeneration of neurons in several brain regions. Effects can be observed from dysfunction of the central nervous system and brain lesions in the hippocampus, the part of the brain associated with memory retention, hence the memory loss characteristic of ASP (Perl et al. 1990, Todd 1993, Doble 2000).

#### **1.4.2. Quantitative analysis of the ASP toxin**

During the first ASP outbreak, the Association of Analytical Chemists (AOAC) mouse bioassay for PSP toxins was used to test extracts of contaminated mussels (AOAC 1990). In response to DA intraperitoneal injection, mice exhibited unique scratching of the shoulders with the hind legs and eventually death (Wright et al. 1989). The limit of detection for this method was approximately  $40 \mu\text{g.g}^{-1}$  of shellfish tissue, inadequate for use in quantitative regulatory determinations.

DA was first identified as the ASP toxin using high performance liquid chromatography (HPLC) and its structure elucidated using mass spectrometry, infrared spectroscopy and nuclear magnetic resonance spectroscopy. For quantitative analysis a rapid extraction technique into methanol:water (1:1 v/v), a clean-up procedure using solid phase extraction (SPE) and analysis by HPLC-UV has been developed (Quilliam et al. 1989, 1995). The clean-up procedure involves passing supernatant, following extraction and centrifugation, through a preconditioned strong anion exchange (SAX) column to eliminate interferences, notably tryptophan, in the HPLC analysis. Many other analytical

techniques have been used and are under development for the rapid detection of DA in seafood.

A semi-quantitative technique for the detection of DA by one-dimensional, thin layer chromatography (TLC) has been developed. Following separation, TLC plates are dried, sprayed with a 1 % solution of ninhydrin and examined under UV light at 254 nm. DA can be observed as a yellow spot (Quilliam et al. 1998). This technique is relatively inexpensive and has a detection limit of approximately 0.5 µg DA.

Capillary electrophoresis (CE) allows the separation of polar compounds and has been used for the quantification of DA in seafood samples. In CE, mixtures of compounds in a sample are separated on a capillary column by the application of an electric field and detected by UV absorbance. This technique has been applied for the analysis of DA and isomers in seafood (Zhao et al. 1997, Gago-Martínez et al. 2003).

#### **1.4.2.1. High Performance Liquid Chromatography (HPLC)**

Despite the development of a range of techniques for quantitative measurement of DA the EU regulatory method for routine detection of DA and epi-DA is High Performance Liquid Chromatography (HPLC) with UV or photo-diode array (PDA) detection (Quilliam et al. 1989, AOAC 2000b). This technique has been formally validated for mussels in an AOAC study (Lawrence et al. 1991) and was the method used for DA analysis in king scallops throughout this research programme.

High performance liquid chromatography is a type of chromatography that employs a liquid mobile phase and a finely divided stationary phase. Reversed phase HPLC with UV or PDA detection allows the fastest and most efficient separations. To achieve good separation of DA and isomers and to suppress ionisation of the carboxyl groups, an acidic mobile phase, commonly a mixture of acetonitrile, deionised water and trifluoroacetic acid, is recommended. Detection of DA and isomers is performed at 242 nm as DA absorbs strongly at this wavelength.

The precise nature of the HPLC column and guard column used for DA analysis varies from laboratory to laboratory, though maintenance of an oven temperature of 40°C is often recommended. Depending on the sensitivity of the detection system, the HPLC-UV detection limit for DA has been estimated at 10-80 ng.ml<sup>-1</sup> (Quilliam et al. 1995). On the other hand, the detection limit of DA in shellfish tissue depends on the extraction and sample clean-up procedure used in the method. If crude extracts are analysed, the practical limit for quantification has been estimated to be 1 µg.g<sup>-1</sup> (Lawrence et al. 1989). Interferences such as the amino acid tryptophan and some of its derivatives are often present in shellfish matrices. Tryptophan has been shown to absorb light at 242 nm and has retention times close to those of DA (Quilliam et al. 1995). Solid phase extraction (SPE) can be used as a part of the sample preparation protocol to concentrate and purify the analyte with a solvent that is compatible with the HPLC. However, with PDA detection systems, differentiation between DA and interferences is straightforward. The reliability of PDA detection systems has allowed the direct analysis by HPLC of crude tissue extracts without SPE clean-up procedures. Gradient elution protocols whereby the composition of the mobile phase is changed during the analysis to achieve improved separations have been developed for the analysis of DA.

In recent years liquid chromatography mass spectrometry (LC-MS and LC-MS-MS) has developed as the dominant technique used for the detection of toxins in shellfish. LC-MS-MS with electrospray ionisation for DA analysis in shellfish samples has been reported (Hess et al. 2001). This method of analysis has the advantage of low detection limits, a high degree of specificity and minimisation of background interferences. LC-MS techniques are under development for the determination of numerous marine toxins in a single sample with one extraction procedure (Quilliam et al. 2001).

As well as these chemical methods for the determination of the ASP toxin, detection of DA in shellfish can also be achieved using immunoassays; both enzyme assays and receptor binding assays are currently in use today. These techniques have several reported advantages over chemical analytical methods including ease of use, rapid results and a higher sensitivity, all useful features in situations where a high sample throughput

is desirable. Diagnostic test kits also have the potential for use in the field, for example on fishing vessels or at aquaculture sites as well as in laboratories.

#### 1.4.2.2. Immunoassays

Immunoassays dependent upon an antigen-antibody reaction have been developed and are available on the market for a range of marine algal toxins. These immunoassays are available in a number of different formats. The enzyme linked immunosorbent assay (ELISA) format for DA is a competitive binding assay where free DA in the sample matrix competes with a plate coated DA-conjugated protein for binding to specific anti-DA antibodies. In a comparative study between ELISA and HPLC techniques for DA analysis; results from the ELISA exhibited a good correlation with results by HPLC, but values were approximately 9 % higher by the ELISA technique (Smith & Kitts 1995). This difference was attributed to the detection of DA isomers in the shellfish sample that were not determined by the HPLC method. Garthwaite et al. (2000) claimed that the ELISA method had detection limits 500 times below the maximum permitted level for DA. Despite these advantages, the immunoassay in the ELISA format currently available on the market (BioSense®) remains a relatively expensive method for the determination of DA in scallops (Bender et al. 2004).

The Jellett Rapid® test kit for ASP was developed to meet the demand for a fast, reliable, accurate, easy to use, cost-effective method that potentially could be used autonomously in the field for the detection of DA in shellfish matrices. The rapid test kit works on the principal of immunochromatography and is available in the lateral flow format. It is recommended as a semi-quantitative method and the detection limit is set at  $10 \mu\text{g.g}^{-1}$  (Jellett et al. 2002). The toxin is extracted from a shellfish tissue sample using the methanol:water extraction, as for the HPLC method, however 10 g of shellfish homogenate is extracted into 40 ml of methanol: water. Once filtered, only 100  $\mu\text{l}$  of sample extract are required for the test. This volume is mixed with buffer and pipetted onto the lateral flow format of the test strip. A visual determination of the presence or absence of DA in the sample is provided. Similar format Jellett kits have been developed

for PSP and DSP toxins and the kits, with adjusted sensitivity, may also have a role in phytoplankton monitoring. Validation studies with the ASP kit performed to date suggest that it may be used as part of a routine monitoring programme (Mackintosh and Smith, 2002).

### 1.5. Regulation of ASP toxin in shellfish

Following the ASP outbreak in Prince Edward Island, Canada, two workshops were held during 1987 to establish a regulatory limit for DA in shellfish. The Health and Welfare Ministry of Canada in 1988 established a regulatory limit of  $20 \mu\text{g.g}^{-1}$  DA in shellfish tissue to protect the public from ASP. This limit of  $20 \mu\text{g.g}^{-1}$  is equal to an intake of 0.1 mg DA/kg body weight per human assuming that body weight is 60 kg and that consumption of mussels is 300 g (Todd 1993).

Detection of DA in mussels from Galicia, Spain in 1994 (Arévalo et al. 1997) and in Portuguese shellfish in 1995 (Vale and Sampayo, 2001) led to the introduction of EU Council Directive 61/97/EC to amend EU Directive 91/492/EEC laying down the health conditions for the production and placing on the market of live bivalve molluscs. Under the amendment a regulatory limit for ASP content in the edible parts of molluscs (the entire body or any part edible separately) must not exceed  $20 \mu\text{g.g}^{-1}$  of DA determined using the HPLC method, similar to the limit established by the Canadian authorities.

Compliance with these Directives resulted in extensive shellfishery closures, perhaps the most significant being those applied to the king scallop fishery off the west coast of Scotland, responsible for considerable financial hardship for scallop fishermen (Gallacher et al. 2001, Smith et al. 2005). Localisation of the majority of toxin in the scallop hepatopancreas, slow depuration rates of toxin from scallops leading to extended periods of closure and high inter-animal variation in toxin concentration led to the introduction of EU Commission Decision 2002/226/EC. This Decision allowed the harvesting of bivalve molluscs belonging to the species *Pecten maximus* and *Pecten jacobaeus* with a concentration of DA in the whole body between  $20 \mu\text{g.g}^{-1}$  and  $250 \mu\text{g.g}^{-1}$  if the parts to be



marketed, principally the adductor muscle and gonad, in the sample contained less than  $4.6 \mu\text{g.g}^{-1}$ . The Decision specified that for determination of the DA concentration of the entire body, analysis should be performed of a homogenate of 10 molluscs and that for determination of the DA concentration of the edible parts, analysis should be performed on a homogenate of 10 individual parts.

### **1.5.1. Shellfish industry and ASP management approaches**

Some Member States in implementing Decision 2002/226/EC have used an area management approach whereby determination as to whether an ICES box or area remains open or closed for fishing depends on the DA concentration from a single sample of 10 scallops. Under this approach, if the DA concentration of the sample is below  $20 \mu\text{g.g}^{-1}$  then the area is open for harvesting, if above  $250 \mu\text{g.g}^{-1}$  then the area is closed for harvesting and if between  $20 - 250 \mu\text{g.g}^{-1}$  scallops from the fishing area must be tested and the edible parts must have a DA concentration below  $4.6 \mu\text{g.g}^{-1}$  before scallop harvesting can commence. Scallops harvested from fishing areas in this intermediate DA category must all be processed and the edible parts must have a DA concentration below  $20 \mu\text{g.g}^{-1}$ . As a practical example, if following analysis of a scallop sample a DA concentration in the whole body tissues of  $19 \mu\text{g.g}^{-1}$  is reported then that fishing area is open for harvesting, if on the other hand a DA concentration of  $21 \mu\text{g.g}^{-1}$  is reported, that fishing area is open for harvesting only if the edible parts of the sample have a DA concentration below  $4.6 \mu\text{g.g}^{-1}$  and under such circumstances all harvested scallops must be processed. Not surprisingly such an approach has caused much consternation among both fishermen and regulators. From a fishing perspective such an approach disregards the high inter-animal variability in DA concentration within an area, which was used as the basis for establishing the  $4.6 \mu\text{g.g}^{-1}$  limit. Given the extensive nature of some fishing areas and the lack of understanding of the factors causing inter-animal variation in scallops, the possibility exists that an area could be closed for harvesting merely because the sample taken for analysis was unrepresentative of the DA concentration in the entire area. From a regulatory perspective, an area-based approach to management necessitates that scallop samples for analysis are procured from each area on a regular basis to allow



fishery management. Implementation of such an approach can only be undertaken if financial supports are available to allow such a sampling and analysis programme.

In Ireland, the Marine Institute under a service contract from the Foods Safety Authority of Ireland (FSAI) undertakes the monitoring programme for all shellfish biotoxins. Two approaches are used for the management of ASP, one for scallops produced by aquaculture and the other for scallops captured from the wild fishery. Shellfish aquaculture in Ireland has expanded rapidly in recent years, production reaching 43,091 tonnes valued at € 43.6 million in 2004 (Parsons 2005). There are currently a total of ninety-six shellfish production areas and the species under cultivation include the mussel *Mytilus edulis*, native oyster *Ostrea edulis*, Pacific oyster *Crassostrea gigas*, Pacific clam *Tapes philippinarum* and king scallop *Pecten maximus*. Scallop production founded on natural seed collection in Mulroy Bay, County Donegal and hatchery seed production by Cartron Point Shellfish Ltd. in County Clare is now almost entirely based around juvenile culture in suspended nets followed by extensive sowing culture on the seabed. Production volumes though limited to date are gradually expanding and amounted to 103 tonnes valued at € 437,000 in 2004 (Figure 3).

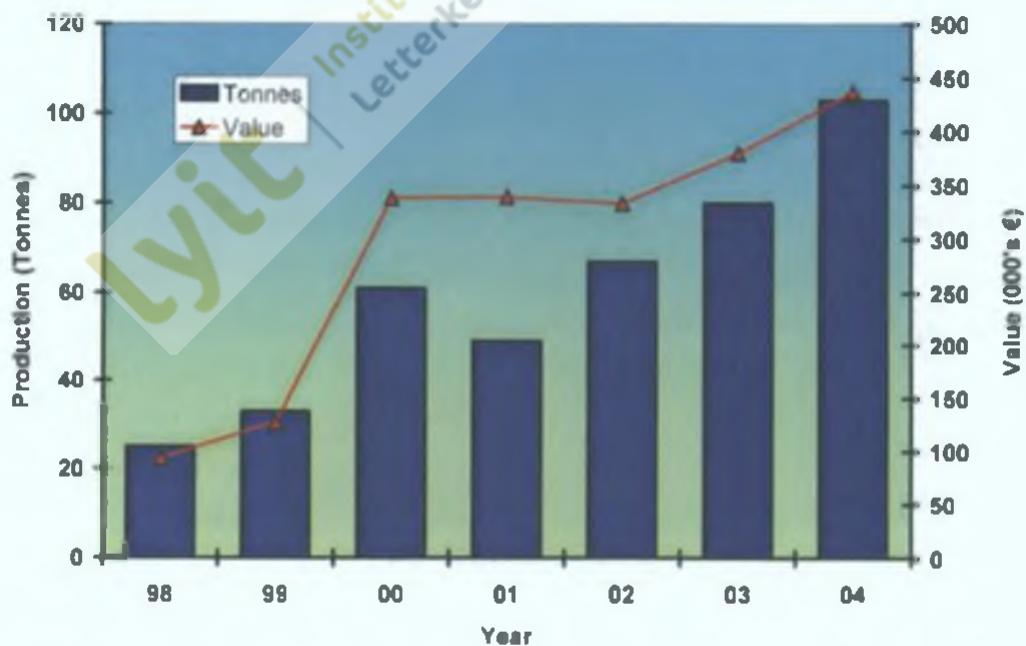


Figure 3. Aquaculture production of king scallop, *Pecten maximus*, 1998-2004.

Cultivated scallops are sampled weekly from defined sampling points in relevant aquaculture bays and managed according to the DA limits prescribed in legislation. Samples must be provided for testing two weeks prior to the commencement of harvesting and must be approved as being suitable for entry into the marketplace. In the event of high DA concentrations, harvesting of cultivated scallops from aquaculture sites can easily be postponed until levels are below  $20 \mu\text{g.g}^{-1}$ . This flexibility in the harvest date to correspond with DA concentrations below the levels prescribed in legislation has allowed cultivated scallops to be marketed in the fresh in-shell sector of the market where premium prices can be attained.

Landings of king scallop, *Pecten maximus*, by the Irish fleet in 2004 totalled over 1,460 tonnes with a value of almost € 3 million. Annual landings of scallops from 1995 – 2004 are provided in Figure 4.

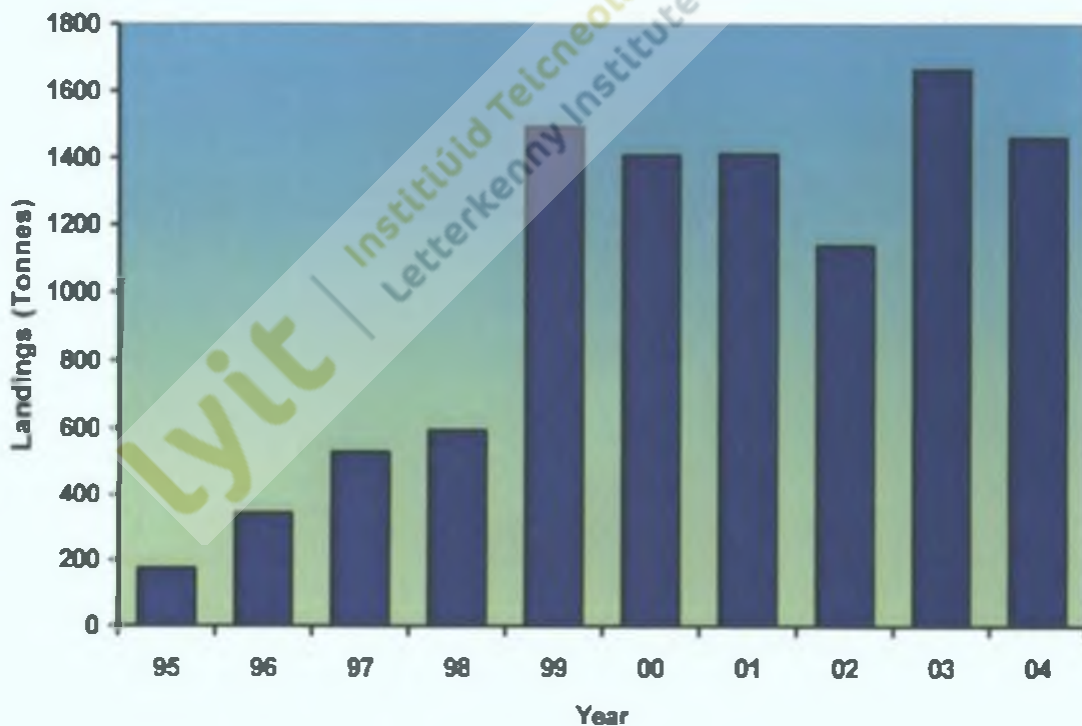
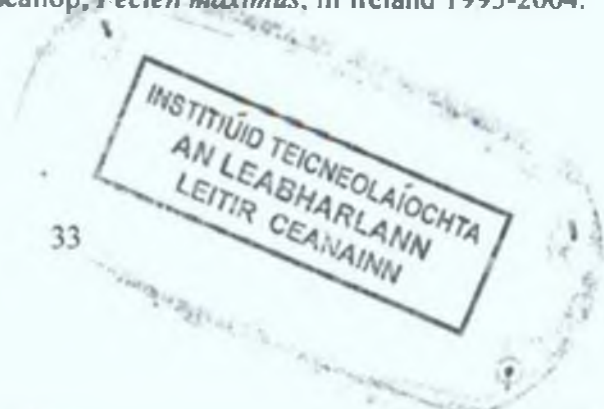


Figure 4. Annual landings of king scallop, *Pecten maximus*, in Ireland 1995-2004.



Since 1999 mean landings have averaged 1,400 tonnes annually. The majority of the scallops landed in Ireland are from the southeast scallop fishery, which underwent significant expansion between 1995 and 2002. Pre-1997 only 103 dredges were being used in the fishery, between 1997 and 2000 this number increased to 198 and in 2002 the number of dredges in use totalled 528 (Tully et al. 2002). Corresponding extension in the fishing grounds covered has also occurred; in 1970 only two small inshore grounds were fished but by 2002 fishing activity was distributed throughout the southern Irish Sea, Isle of Man, western English Channel and northwest of Brittany (Irish Stock Book, 2005). Despite these substantial increases in fishing effort and the dredging of more distant grounds, landings have remained relatively stable since 1999 suggesting serious difficulties for the industry in the future. The unit value of €2,000 per tonne in 2003 and 2004 represented a 40 % reduction on the value in 2001 and was the lowest price to have occurred since 1992. It has been suggested that this reduction in value arose as a consequence of the long steaming time between fishing grounds and the market causing the landed product to be in poor condition (Tully et al. 2002).

A landings-based approach is used for the management of ASP in scallops from the wild fishery and necessitates that all the catch be shucked to remove the hepatopancreas and then frozen prior to testing. No product is allowed to enter the marketplace before test results indicate that the DA concentration in adductor and gonad is below  $20 \mu\text{g.g}^{-1}$ . This landings-based approach to scallop fishery management has allowed resumption of harvesting from fishing grounds that otherwise would have remained closed under amendment 97/61/EC to Directive 91/492/EEC, but has necessitated that all the catch be processed prior to sale. This distinction in biotoxin management between cultivated and wild scallops has caused some concern amongst scallop fishermen based around the lack of access to the high value fresh market for wild fishery scallops. This market restriction has impacted most severely in those regions off the southeast coast of Ireland in which scallop fisheries expanded in scale and importance during the 1990's.

## **1.6. Variability in DA concentration**

Clearly despite the different approaches used to implement regulations for ASP management, of utmost significance is the reported DA concentration in the whole shellfish or tissue under investigation. Tracing back from the reported DA concentration, uncertainty in the measurement can arise during the analytical stage in the laboratory, during storage and transportation from the field and as a result of unrepresentative sampling in the field. It is generally accepted that the greater the number of factors influencing a stage or the greater the number of steps involved in a procedure, the greater the potential for uncertainty in the measurement.

### **1.6.1. Uncertainty arising in the laboratory**

In the laboratory uncertainty in the measurement can arise during the preparation of tissues for analysis, during the toxin extraction procedure, at the sample clean-up stage if one is included in the procedure and also at the toxin measurement stage.

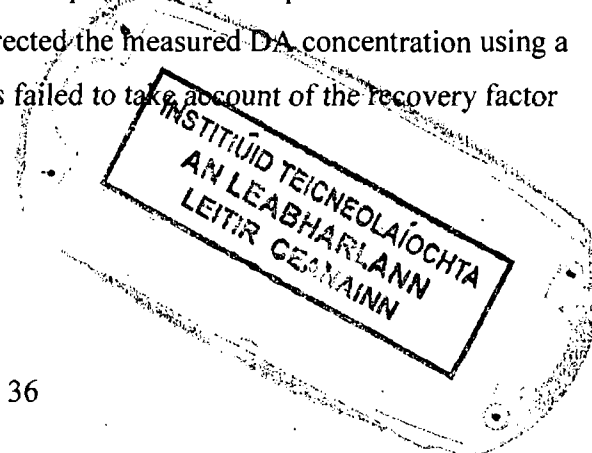
With regard to uncertainty arising at the tissue preparation stage, significant differences in scallop gonad toxicity have been reported in a comparative study of four different tissue preparation techniques. Variations in toxin concentration were attributed to differences in the contribution of the digestive loop, which passes through the gonad in this species, in each of the sample preparation techniques. The significant variation in toxin concentration demonstrated the need for a standardized tissue preparation technique for the analysis of scallop gonads (Hess et al. 2000, Campbell et al. 2003).

At the toxin extraction stage variation in both the number of extractions and the extraction solvent have been reported. Although exhaustive extraction is recommended for situations where knowledge of the total DA present is required, e.g. the preparation of Reference Materials, time constraints imposed on regulatory agencies often necessitate the use a single-step extraction procedure (Quilliam et al. 1995). Using a single-step extraction procedure, the efficiency of extraction varied from 72-100 % depending on

both the scallop tissue under investigation and the concentration of DA present (Harkin et al. 2004). The most common extraction solvent used for DA extraction from shellfish tissue comprises aqueous methanol (1:1, v/v) (Quilliam et al. 1995, Ferdin et al. 2002, James et al. 2005, Smith et al. 2006). Other extraction solvents have been reported in the literature including 0.1 M hydrochloric acid (Lawrence et al. 1989), water:methanol (1:2, v/v) (Powell et al. 2002) and water:methanol (1:4, v/v) (Vale et al. 2002a).

Numerous studies have been conducted to determine the effect of a range of conditions on the stability of DA following extraction. DA extracts were stable in acetonitrile and deionised water (1:9 v/v) following extraction for up to one year when stored in the dark at  $-12^{\circ}\text{C}$  (Ravn 1995). In sterile saline solutions, both DA and its isomers were stable at ambient temperatures, even when exposed to light (Johannessen 2000). By contrast aqueous solutions of DA showed extensive degradation when exposed to high temperatures, light or various pH's, even when stored at  $-12^{\circ}\text{C}$  (Bates et al. 2004). The stability of refrigerated DA extracts may depend on the type of tissue under analysis. DA concentration of scallop gonad extracts in aqueous methanol decreased by approximately 45 % compared to a decrease of 15 % with whole scallop extracts over a two-week period, although extracts were more stable when stored refrigerated in citrate buffer (Smith et al. 2006). To minimise toxin degradation, it is now recommended that aqueous solutions containing DA be stored under argon or nitrogen, in the dark and refrigerated. For long-term storage of aqueous DA solutions, it is recommended that samples should be stored at  $-80^{\circ}\text{C}$  (Quilliam 2003).

Performance assessments of the DA measurement procedure on a range of tissue samples, crude extracts and standard solutions by Irish and UK laboratories highlighted the efficiency of the SAX cartridges used in sample clean-up as a potential source of variation in results. Some laboratories corrected the measured DA concentration using a cartridge recovery factor, other laboratories failed to take account of the recovery factor (Hess et al. 2005).





Numerous high performance liquid chromatography (HPLC) methods have been developed for determination of the DA concentration featuring a range of detection systems. The validated technique of Lawrence (1989) subsequently modified by Quilliam (1995) used UV-absorption for the detection of domoic acid. Further HPLC methods using chemical derivatization of the toxin and fluorescence detection (Wright et al. 1995), photodiode-array UV detection (Hess et al. 2001, Hess et al. 2005, James et al. 2005, McCarron et al. 2006) and mass spectrometric (MS and MS/MS) detection (Lawrence et al. 1994, Campbell et al. 2001, Furey et al. 2001, Hess et al. 2005, James et al. 2005, Lopez-Rivera et al. 2005) have all been developed. There was no difference in the quantification of DA in standard solutions or shellfish using either LC-UV or LC-MS detection (Hess et al. 2005).

### **1.6.2. Uncertainty arising in the storage and transportation**

When king scallop samples were stored for 2-3 days at 12 °C, significantly higher toxin concentrations were detected in gonad tissues compared to samples stored at 4 °C and analysed within 48 hr (Smith et al. 2006). With tissue samples frozen for 1-month, reduced concentrations of toxin were attributed to slow decomposition of DA (Vale and Sampayo, 2002). On the basis of these reports it would appear that uncertainty in monitoring results might arise if the effects of either short-term or long-term storage conditions on DA concentration in scallop tissues are ignored.

### **1.6.3. Uncertainty resulting from unrepresentative field sampling**

Variations associated with the analytical steps of the procedure were reported to be small by comparison with inter-animal variation in the field (Fryer et al. 2002). Significant variation in DA concentrations between individual *Pecten maximus* and their body components, might result in samples that are unrepresentative of the area as a whole and was suggested as a factor that complicates the management of scallop fisheries during ASP events (Campbell et al. 2001).

DA accumulation, prolonged retention of the toxin and slow rates of depuration in king scallop, *Pecten maximus*, have been widely reported. Many of these investigations also reported high levels of inter-animal variability in toxin concentration between scallops in the same sample (Arévalo et al. 1998, Gallacher 2001, Campbell et al. 2001, Blanco et al. 2002, Fryer et al. 2002, FSA 2001, James et al. 2005). As an example of the extent of this inter-animal variation in DA concentration at a single sampling site, coefficients of variation (CV) in tissues excluding the adductor and gonad ranging from 29 % to 120 % and in gonad ranging from 45 % to 85 % have been reported. Significant differences in toxin concentrations between samples collected only 25 metres apart occurred (Campbell et al. 2001). Such results provide an indication of the scale on which microhabitat differences may influence ASP toxicity in *P. maximus* populations and the difficulties faced by regulators in managing scallop fisheries. Similar high inter-animal variations in DA toxin concentrations have been reported in razor clams (Wekell et al. 2002) and likewise with PSP toxins in sea scallops, *Placopecten magellanicus* (Cembella et al. 1994).

Exposure of scallops to toxic *Pseudo-nitzschia* cells is now accepted as the major factor responsible for the presence of DA toxin. Numerous physical and biological factors have been suggested to account for the high inter-animal variability between shellfish. Physical factors suggested as contributing to inter-animal variation include: water temperature (Silvert et al. 1992, Morono et al. 2001); water currents, upwelling and water depth (Cembella et al. 1993, Bricelj et al. 1998, Curtis et al. 2000). Biological factors that may play a role include: filtration rate and volume specific toxin concentration (Morono et al. 2001); duration of exposure to toxic cells (Bricelj et al. 1998); differences in feeding rates among individuals (Cembella et al. 1993); food availability at different water depths (Curtis et al. 2000, FSA 2001, Wekell et al. 2002, Haya et al. 2003); differences in feeding due to bloom patchiness (Bricelj & Shumway 1998, Haya et al. 2003); reproductive status (Cembella et al. 1994, Costa et al. 2005); body weight and shellfish size (Aalvik et al. 1981, Arévalo et al. 1998, Powell et al. 2002, Strohmeier et al. 2005, Costa et al. 2005). It has also been reported that toxic *Pseudo-nitzschia* cells descend after a bloom and can remain toxic at depth (Dortch et al. 1997, Trainer et al.

1998, 2000) and this may also account for some of the variability between scallops. The fact that dense *Pseudo-nitzschia* blooms do not always coincide with elevated concentrations of DA in shellfish suggests that factors influencing the toxin production of algal cells may also have a role in inter-animal variability (Marchetti et al. 2004).

Despite the importance of inter-animal variation in the field in potentially contributing to uncertainty in the reported DA concentrations, limited attention has been addressed to studies of the significance of the above factors in causing such variation.

### **1.7. Objectives of this research programme**

Given the limited information available on variation in DA concentrations in the field, particularly in Irish waters and the factors that might influence such variability, the objectives of the programme were:

- To determine the distribution of DA between different body tissues of the king scallop and the extent of inter-animal variation in a range of inshore and offshore sites.
- To investigate variations in DA concentration as a function of scallop shell size, body weight and age in a range of inshore and offshore sites.
- To investigate variation in DA concentration as a function of water depth in a range of inshore and offshore sites.
- To investigate the uptake of non-cellular DA by king scallops
- To compare HPLC, the BioSense® ELISA kit and the Jellett Rapid® ASP kit for the determination of DA with the objective of making an assessment of the value of such kits for rapid ASP monitoring in Irish waters.



The work programme undertaken to meet these objectives includes:

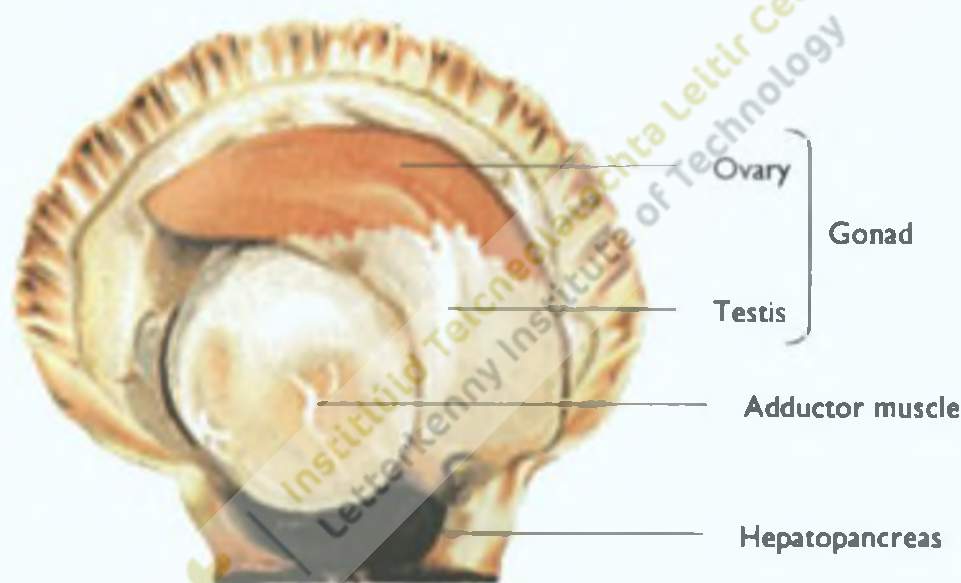
- Establishing a protocol for the HPLC determination of DA at Letterkenny Institute of Technology using Certified Reference Standards, Certified Reference Material, Laboratory Reference Material and systems for data collection and analysis to ensure quality assured results throughout this research programme.
- Monthly determination over a 12 month period of the DA concentration in hepatopancreas, gonad and adductor of four different size groups of king scallop from a seabed aquaculture site in Clew Bay, County Mayo. Variations in DA concentration with scallop size were also investigated at six sites around the Isle of Man on three sampling dates, at five sites off the southeast coast on one sampling date, at one site in Strangford Lough on two sampling dates and at one site in Kilkieran Bay on one sampling date. Toxin concentration in the hepatopancreas was measured on an individual basis at all sites to provide data on inter-animal variation in DA concentration between sites.
- Monthly determination over a two-year period of DA concentration in small-sized scallops from an inshore site in Clew Bay, County Mayo for comparison of toxicity data with cell counts of *Pseudo-nitzschia* species in phytoplankton.
- Monthly determination over a period of one-year of DA concentration in seabed and suspended scallops of uniform size from an inshore site in Clew Bay, County Mayo to assess the toxin concentration of scallops both on the seabed and suspended in the water column. Toxin concentration in the hepatopancreas and gonad were measured on an individual basis in all scallops to provide data on inter-animal variation in DA concentration of seabed and suspended scallop.
- Two investigations of the spatial variation in DA concentration of commercial-sized king scallop from within an area approximately the size of a single ICES fishing box. The aim of this study was to determine the extent of variation in DA

concentration within a restricted area and to investigate variation in DA concentration with water depth. Supporting studies of spatial variation in DA concentration within a restricted area were also performed at six sites around the Isle of Man on three sampling dates. The Isle of Man investigations and a further study in Strangford Lough provided further data on the variation in toxin concentration with water depth. Toxin concentrations in the hepatopancreas were measured on an individual basis in all samples to provide data on inter-animal variation in DA concentration.

- An investigation of the uptake of non-cellular DA by scallops in the presence and absence of non-toxic algae. This trial was conducted to determine if scallops held in tanks could absorb DA from seawater. Such evidence might provide some explanation of how scallops might become intoxicated, even in the absence of toxic *Pseudo-nitzschia* cells.
- Two investigations comparing first the HPLC and the BioSense® ELISA kit and secondly the HPLC and the Jellett Rapid® ASP kit for the determination of DA in scallop tissue samples to assess both kits technically and practically in terms of their accuracy, precision, sensitivity, ease of use and cost for rapid ASP monitoring in Irish waters.

## 2. Variation in DA concentration with scallop organ

The monomyarian scallops have an anatomy unlike that of other commercial bivalve groups such as the mussels, cockles, clams and oysters. Although all the soft parts of the king scallop, *Pecten maximus*, are edible, in Europe the adductor muscle and the gonad are the parts generally consumed. The gonad is largely separate from the rest of the visceral mass allowing for easy removal and analysis of the organ (Beninger and LePennec 1991). The anatomical parts considered in terms of their DA concentration are illustrated in Figure 5.

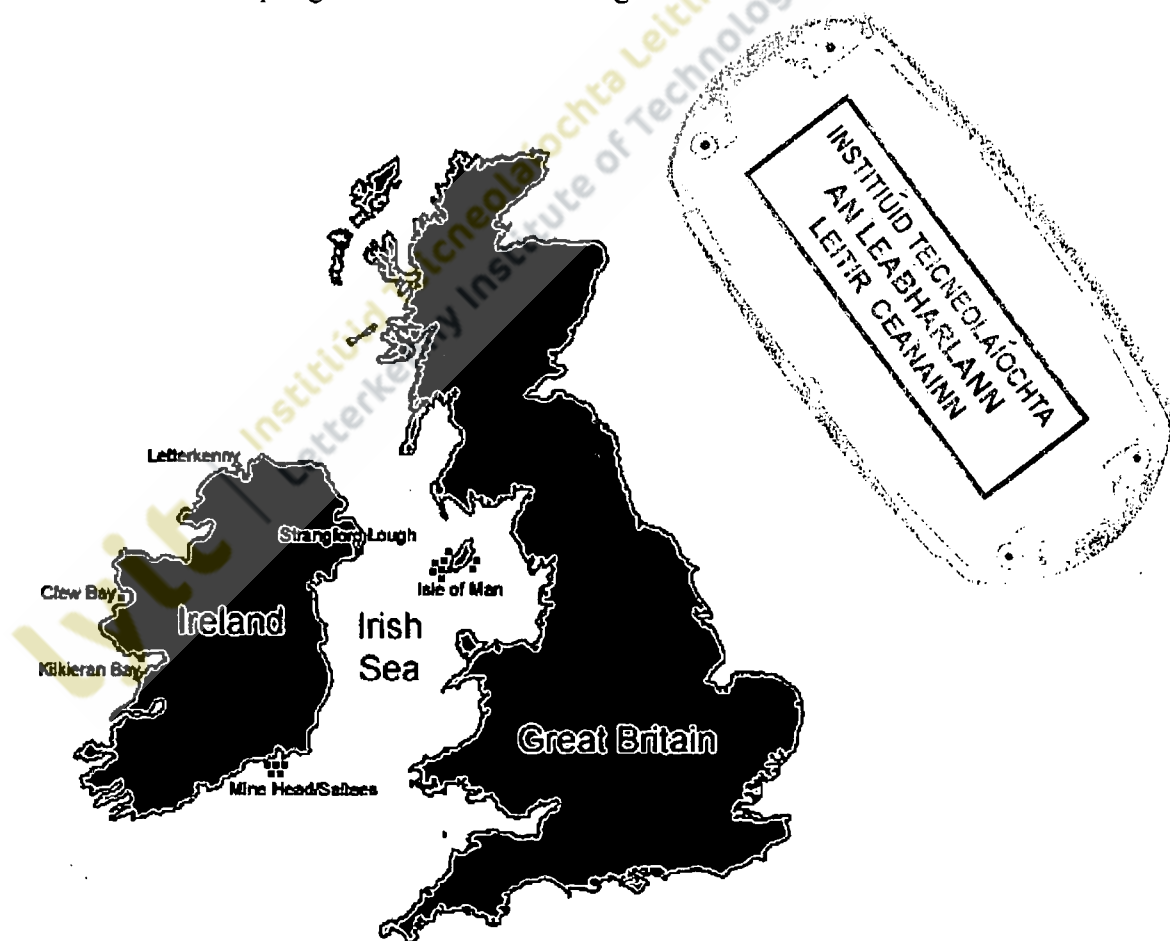


**Figure 5.** Internal anatomy of the king scallop, *Pecten maximus*, with the left shell valve and mantle removed to reveal the hermaphrodite gonad.

Considerable variation in DA concentration between the body components of king scallop, *Pecten maximus*, have been reported. Highest ASP toxin concentrations were recorded in the hepatopancreas, significant amounts in gonad and much lower concentrations, if any, detected in the adductor muscle (Arévalo et al. 1998, Hess et al. 2000, Campbell et al. 2001, Gallacher et al. 2001, FSA 2001, Fryer et al. 2002, James et

al. 2005, Smith et al. 2006). In terms of the relative toxin distribution between the different tissues, estimates vary for the proportion of toxin in the edible parts, the gonad and adductor muscle, from 1 % to 6 % of the total DA burden (Blanco et al. 2002, Campbell et al. 2003).

In this research programme the distribution of DA toxin has been investigated in the tissues of king scallops from Clew Bay, County Mayo (Paper 1, 3, 5), from the Saltees and Mine Head fishing grounds off the southeast coast of Ireland (Paper 2, 5, 6), from fishing grounds around the Isle of Man (Paper 4, 5), from Strangford Lough, County Down, Northern Ireland (Paper 5) and Kilkieran Bay, County Galway (Paper 5). The location of these different sampling sites is illustrated in Figure 6.



**Figure 6.** King scallop sampling sites in this research programme.

As anticipated, the DA concentration in king scallop was highest in the hepatopancreas, followed by the tissue remainder (mantle, gills, kidneys etc.), gonad and adductor muscle (Paper 1, 2, 3, 4, 5, 6). Similar distribution of the toxin burden in scallops has been reported in other studies (Douglas et al. 1997, Arévalo et al. 1998, Hess et al. 2000, Campbell et al. 2001, Gallacher et al. 2001, FSA 2001, Fryer et al. 2002, James et al. 2005, Smith et al. 2006). The highest concentration of DA recorded in hepatopancreas of an individual scallop was 3834.4  $\mu\text{g.g}^{-1}$  from Strangford Lough (Paper 5).

Concentrations in hepatopancreas of this magnitude are consistent with, though slightly higher than, the values quoted in the literature: 3689  $\mu\text{g.g}^{-1}$  (Campbell et al. 2001), 2083  $\mu\text{g.g}^{-1}$  (Arévalo et al. 1998), 2820  $\mu\text{g.g}^{-1}$  (James et al. 2005). By contrast, the lowest DA concentrations in hepatopancreas from fishing grounds on the east of the Isle of Man were below the limit of quantification by HPLC (Paper 4). Given the very slow depuration rates reported in king scallop (Arévalo et al. 1997, Gallacher et al. 2001, Blanco et al. 2002, Smith et al. 2006) such low DA concentrations, recorded from both sites on the east of the Isle of Man, suggested limited exposure of scallop from these fishing grounds to DA toxin (Paper 4). Maximum and minimum DA concentrations in hepatopancreas and gonad samples recorded in this research programme are presented in Table 2.

Location	DA concentration ( $\mu\text{g g}^{-1}$ )						Reference
	Hepatopancreas		Gonad		Adductor muscle		
	Max	Min	Max	Min	Max	Min	
Clew Bay, County Mayo	1348.2	16.6	9.18	LOQ	LOQ	LOQ	Paper 1, 5
Saltees/Mine Head fishing grounds	913.4	13.9	61.2	2.02	31.8	LOQ	Paper 2, 5
Clew Bay, County Mayo	1212.6	25.8	8.45	1.96	LOQ	LOQ	Paper 3, 5
Isle of Man fishing grounds	558.6	LOQ	29.5	LOQ	7.3	LOQ	Paper 4, 5
Strangford Lough, County Down	3834.4	186.9	52.4	8.5	7.7	LOQ	Paper 5
Kilkee Bay, County Galway	78.2	22.6	LOQ	LOQ	LOQ	LOQ	Paper 5

**Table 2.** Maximum and minimum DA concentrations in individual hepatopancreas, composite gonad and composite adductor muscle samples from locations around Ireland and the Isle of Man. LOQ = Limit of quantification.

Quantification of the variation in toxin concentration among individuals of the same species from the same area and an understanding of the causes of such variation are important for the development of reliable sampling and management protocols. DA concentrations in hepatopancreas of king scallop exhibited high inter-animal variability within a sample. In terms of the co-efficient of variation (CV), a measure of spread often used to compare levels of variation across populations with different mean concentrations, inter-animal variability in DA concentration in samples comprising twelve hepatopancreas analysed individually ranged from 12.5 % to 82.5 % in Clew Bay (Paper 1), from 24 % to 65 % in the Saltees/Mine Head fishing grounds (Paper 2), from 17.8 % to 55.5 % in Clew Bay (Paper 3) and from 16.1 % to 70 % in the Isle of Man fishing grounds (Paper 4). The ranges in CV's of sample batches from these sites were considerably higher than the values of 27.6 % to 43.9 % from Kilkieran Bay (unpublished) and 11.0 % to 27.3 % from Strangford Lough, (unpublished). The reduced ranges in CV at these latter sites were attributed to the single diver-sampling occasion at one site in Kilkieran Bay and the two diver-sampling occasions at one site in Strangford Lough. In summary, inter-animal variability in hepatopancreas ranged from 12.5 % to 82.5 % over the entire research programme. Similar high levels of inter-animal variability in toxin concentration in king scallop samples have been widely reported (Arévalo et al. 1998, Campbell et al. 2001). Fryer et al. (2002) reported CV's of DA concentration in whole scallops ranging from 18 % to 68 %, less than those of gonad and adductor muscle that ranged from 41 % to 140 % and from 57 % to 122 % respectively. Since, in this research programme, both gonad tissues and adductor tissues were analysed as composite samples of each tissue, comparisons of the levels of inter-animal variability between these tissues were not possible.

In the Clew Bay temporal study over a one-year period (Paper I), the CV's of DA concentration in hepatopancreas in small-sized scallops ranged from 22.7 % to 82.5 % and were significantly larger than in the other three size groups; 15.0 % to 46.4 % in medium scallops, 17.8 % to 49.5 % in large scallops, 12.5 % to 40.3 % in very large scallops, indicative of the more variable mean DA concentration in smaller-sized scallops.

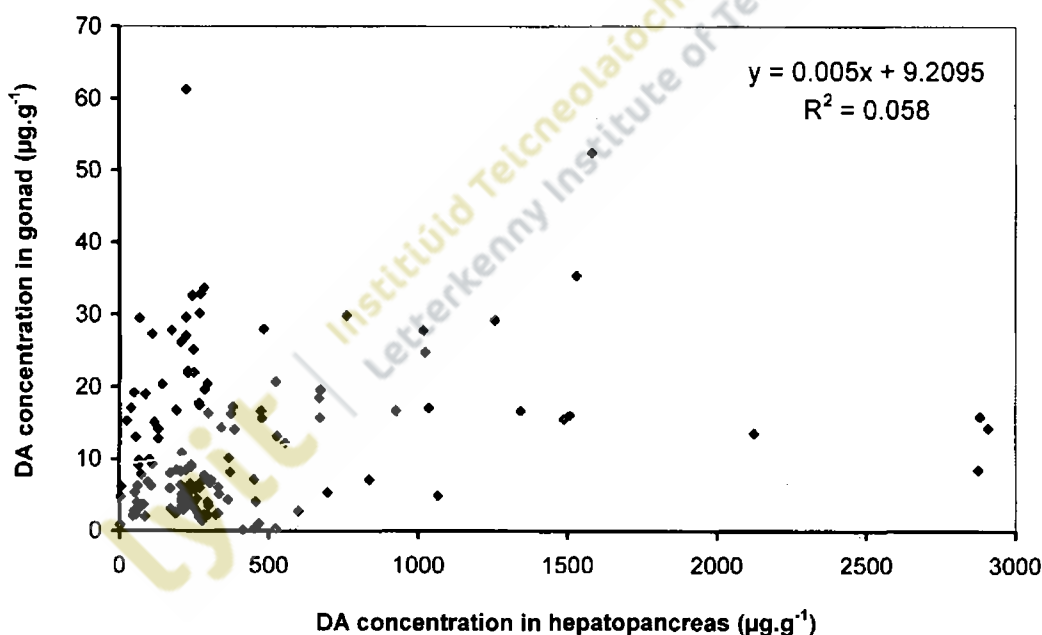


The principal factor responsible for the presence of DA in king scallops is now accepted to be exposure to toxic *Pseudo-nitzschia* cells. Variability in the toxin concentration in hepatopancreas between individual scallops (Paper 1, 2, 3, 4, 5, 6) and between different size groups of scallop (Paper 1, 2, 4, 5) may be caused by differences in the rate of feeding on toxic cells. Rapid DA accumulation has been reported in both the bay scallop, *Argopecten irradians*, fed toxic *P. multiseriata*, accumulating DA in the hepatopancreas to levels of  $60 \mu\text{g.g}^{-1}$  within eighty-four hours and the sea scallop, *Placopecten magellanicus*, incorporating DA within twenty-four hours of exposure to toxic cells (Douglas et al. 1997). Differences in the rates of DA depuration between individual scallops, reported as being slow in king scallops, may also influence inter-animal variability in toxin concentration (Arévalo et al. 1997, Blanco et al. 2002).

Maximum and minimum DA concentrations in composite gonad samples from this research programme are provided in Table 2 and are consistent with values reported in the literature (Arévalo et al. 1998, Hess et al. 2000, Campbell et al. 2001, 2003). In Clew Bay toxin concentrations in composite gonad tissue never exceeded the regulatory limit of  $20 \mu\text{g.g}^{-1}$  on any sampling occasion (Paper 1, 3) but were exceeded at seventeen of the sixty-nine stations off the southeast coast of Ireland (Paper 2), with the highest DA concentration in a composite gonad sample of  $61.2 \mu\text{g.g}^{-1}$  recorded. DA concentrations in composite gonad samples from the Isle of Man exceeded the regulatory limit in seven of the twenty-two samples and exhibited a maximum concentration of  $29.5 \mu\text{g.g}^{-1}$  (Paper 4). DA concentration in composite gonad samples from Strangford Lough, Northern Ireland reached as high as  $52.41 \mu\text{g.g}^{-1}$  (unpublished data) and was below the limit of quantification by HPLC in composite gonad samples from Kilkieran Bay, County Galway (unpublished data). In summary concentrations of DA in composite gonad samples ranged from below the limit of quantification by HPLC to a maximum of  $61.2 \mu\text{g.g}^{-1}$ , slightly lower than the maximum values of  $69.9 \mu\text{g.g}^{-1}$  reported by Arévalo et al. (1998) and  $75.47 \mu\text{g.g}^{-1}$  reported by Campbell et al. (2001). In a summary of results from EU toxin testing laboratories, gonad DA concentrations were generally less than  $120 \mu\text{g.g}^{-1}$  with a reported maximum value of  $283 \mu\text{g.g}^{-1}$  (Fryer et al. 2002).



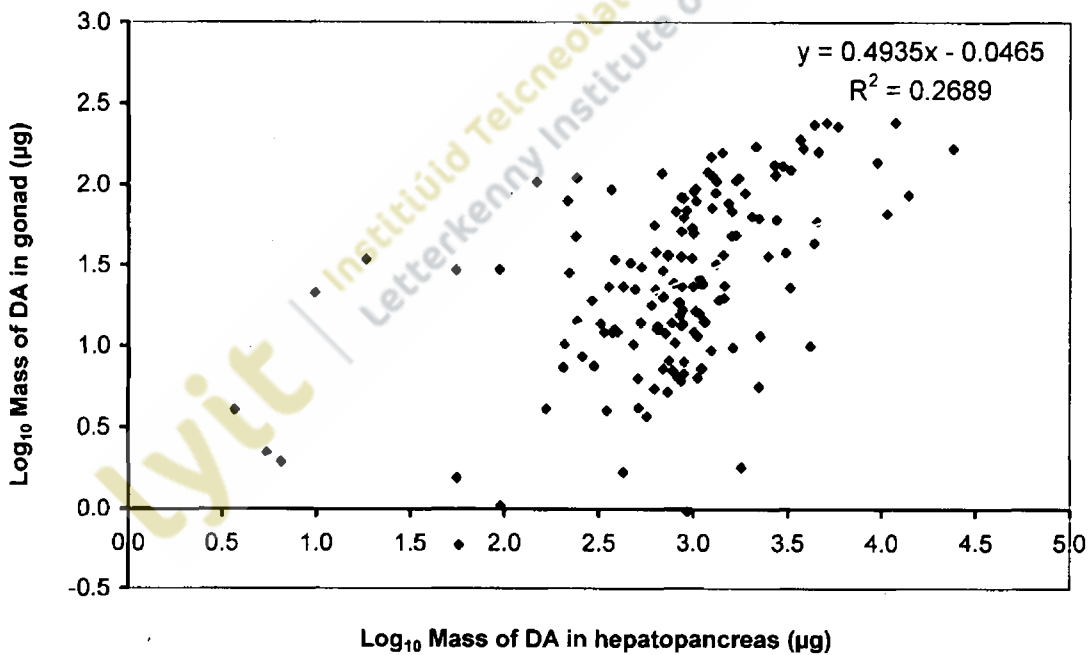
The majority of DA toxin in the gonad is introduced through a digestive loop of intestine passing through this organ in pectinid species (Purchon 1977). In *Pecten maximus* this loop has been estimated to represent up to 6 % of gonadal volume (Mackie, 1986). As a consequence, given the high DA concentrations that occur in the hepatopancreas, there exists via the digestive loop, a potential mechanism for DA contamination of gonad tissue (Hess et al. 2000, Campbell et al. 2003). On the basis that the hepatopancreas represents the source of DA in the gonad, then it might be expected that a relationship would exist between DA concentration in the hepatopancreas and DA concentration in the gonad, though latter concentrations would be substantially reduced by the dilution effect of gonadal tissue. The relationship between mean concentration of DA in hepatopancreas and mean concentration of DA in composite gonad based on the data collected in this research programme is provided in Figure 7.



**Figure 7.** The relationship between the mean DA concentration in the hepatopancreas and the DA concentration in composite gonad samples (n = 157).

Significant changes in gonad size in terms of the relative gonad height (RGH) during the scallop reproductive cycle were recorded during the Clew Bay study (Paper 3). Increases

in RGH as gonad maturation progressed followed by a decreased RGH at spawning resulted in difficulties in understanding the changes in DA concentration in gonad tissues (Paper 3). To overcome the dilution effect by gonadal material and the small seasonal changes in hepatopancreas weight the relationship between the mass of DA in both the gonad and hepatopancreas was considered (Paper 3). Changes in the mass of DA in gonad over the 12-month study period exhibited a similar trend to that for both the concentration and mass of DA in hepatopancreas and suggested that the mass of DA in the gonad could be influenced by the toxin content of the hepatopancreas (Paper 3). Using a similar approach with the entire dataset from this research programme, the relationship between the mass of DA in both the gonad and hepatopancreas was examined. Because of the large spread of data, ranging from 3.69  $\mu\text{g}$  to 24,373  $\mu\text{g}$  of toxin in the hepatopancreas, all data were  $\log_{10}$  transformed. The relationship between the  $\log_{10}$ -transformed data is provided in Figure 8.



**Figure 8.** The relationship between the  $\log_{10}$  mass of DA in the hepatopancreas and the  $\log_{10}$  mass of DA in composite gonad samples ( $n = 157$ ).

The relationship exhibited a highly significant but weak correlation between the  $\log_{10}$  transformed mass of DA in the hepatopancreas and the  $\log_{10}$  transformed mass of DA in composite gonad samples ( $F_{1,155} = 57.02$ ,  $p = 0.0001$ ,  $R^2 = 0.27$ ). The widespread distribution of data and consequent weak correlation was attributed to high inter-animal variability in the toxin concentration and mass of the gonad. High variability in DA concentration in gonad tissue of king scallop has been widely reported and presented difficulties in understanding trends in toxin concentration in scallop gonad samples (Arévalo et al. 1998, Hess et al. 2001, Campbell et al. 2001, 2003, Fryer et al. 2002).

Of the king scallop tissues analysed throughout this research programme, adductor muscle exhibited the lowest concentrations of DA, typically one or two orders of magnitude below that of the hepatopancreas (Paper 1, 2, 3, 4, 5). These findings were consistent with those reported in the literature with *Pecten maximus* (Arévalo et al. 1998, Gallagher et al. 2001, Campbell et al. 2001, Blanco et al. 2002) and *Placopecten magellanicus* (Douglas et al. 1997). Maximum and minimum DA concentrations in composite adductor muscle samples from the study locations in this research programme are provided in Table 2. The DA concentration in forty-nine composite adductor muscle samples from Clew Bay collected on ten sampling occasions over a 12-month period never exceeded the limit of quantification ( $\text{LOQ} = 1.00 \mu\text{g.g}^{-1}$ ) (Paper 1, 3). Concentrations of DA in twenty-two composite adductor muscle samples from the Isle of Man fishing grounds collected on three sampling occasions over a 12-month period exhibited low levels of toxin, the highest DA concentration recorded being  $7.3 \mu\text{g.g}^{-1}$  (Paper 4). A similar maximum DA concentration of  $7.7 \mu\text{g.g}^{-1}$  was reported following analysis of eight composite adductor muscle samples collected on two sampling occasions in Strangford Lough, County Down (unpublished data). The DA concentration in four composite adductor muscle samples from Kilkieran Bay, County Galway never exceeded the limit of quantification ( $\text{LOQ} = 1.00 \mu\text{g.g}^{-1}$ ) (unpublished data). In a study of toxin levels in king scallops from the west coast of Scotland, concentrations of DA in adductor muscle that similarly never exceeded the regulatory limit of  $20 \mu\text{g.g}^{-1}$  were also reported (Campbell et al. 2001). DA toxin concentrations in composite adductor muscle samples of king scallop dredged from sixty-nine stations off the southeast coast of Ireland

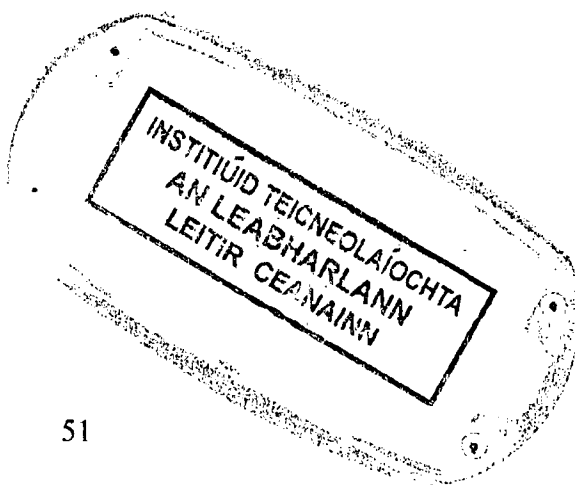
exceeded the regulatory limit at three sampling sites, the maximum DA concentration recorded was  $31.8 \mu\text{g.g}^{-1}$  (Paper 2). In a summary report of 700 analyses of individual ( $n = 489$ ) and composite ( $n = 211$ ) adductor muscle samples by EU toxin testing laboratories, the highest DA concentration recorded in a pooled adductor muscle sample was  $47 \mu\text{g.g}^{-1}$ . Only 1.6 % and 1.9 % of the individual and pooled samples respectively exceeded the regulatory limit of  $20 \mu\text{g.g}^{-1}$  (Fryer et al. 2002).

High levels of PSP toxins in adductor muscle have been reported on occasions in *Placopecten magellanicus* (Jamieson & Chandler 1983). Drum et al. (1993) reported that the Pacific razor clam, *Siliqua patula*, accumulated DA principally within muscular tissue. Since the adductor muscle is not associated with the digestive system or stomach, unlike the gonad, explanations for occasional high levels of toxin in this tissue are presently uncertain. Fryer et al. (2002) proposed that DA in the adductor muscle might arise from the scallop's circulatory fluids or from contamination of the tissue as a result of leakage from the hepatopancreas during storage and also possibly during routine dissection. It was suggested that this contamination might be enhanced during freezing and subsequent thawing of the whole scallops prior to dissection (Fryer et al. 2002). Such a hypothesis regarding contamination of adductor muscle may help to explain the high levels of DA in samples from off the southeast coast of Ireland since these scallops were frozen in-shell and stored in a commercial freezer prior to dissection (Paper 2).

As with the hepatopancreas and gonad, high inter-animal variability of DA concentration in adductor muscle has also been reported. Individual analysis of adductor muscles from king scallop exhibited CV's of 247 % (Campbell et al. 2001). Since only composite adductor muscle samples were analysed in this research programme, comparative results for this tissue are not available (Paper 1, 2, 3, 4). In the study of 69 sampling stations off the southeast coast of Ireland, a CV of DA concentration in composite adductor muscle between sampling sites of 106.2 % was reported (Paper 2), greater than the CV's recorded between sites for hepatopancreas and gonad tissue. Because of the addition of an extra variable in terms of different sampling sites, the values for the CV in adductor muscle are not directly comparable, however all the sampling stations were within a

restricted area less than the size of a single ICES fishing box (Paper 2). The higher variability in DA concentrations in adductor muscle compared to the other tissues may be caused by variability in the toxin concentration within this organ and may be compounded by different levels of contamination during freezing/thawing and during routine dissection (Campbell et al. 2001). Given the low levels of DA recorded in adductor muscles in this study and reported elsewhere in the literature, only minor contamination due to leakage from the hepatopancreas could result in elevated DA concentrations in adductor muscle samples and higher inter-animal variability. Since the adductor muscle, either roe-on or roe-off, comprises an important processed product from this fishery, further work is required to ascertain the extent of contamination from hepatopancreas leakage during storage and during routine dissection.

In summary, the presence of DA toxin in king scallop, *Pecten maximus*, has been clearly documented (Paper 1, 2, 3, 4, 5, 6). Toxin distribution between different body tissues was consistent with literature reports, higher values occurring in hepatopancreas, lower values in gonad and even lower levels in adductor muscle. High inter-animal variability in DA concentration was recorded in hepatopancreas and was suggested in both gonad and adductor muscle. Significant variation in DA concentration between king scallop from the same collection area has also been reported by toxin monitoring programmes elsewhere and has proven problematic for the management of scallop fisheries (Arévalo et al. 1998, Gallacher et al. 2001, Campbell et al. 2001, FSA 2001, Fryer et al. 2002, Smith et al. 2006). An understanding of the factors affecting inter-animal variability in shellfish is imperative for the development of reliable sampling and management protocols for DA outbreaks.



### 3. Variability in DA concentration with scallop size

Under EU regulations (Annex XII of 850/98) the minimum landing size of king scallop has been established as 100 mm shell length, a size usually attained after 3 to 4 years of growth in the southeast scallop fisheries (Tully et al. 2002). The largest scallop provided for analysis during this research programme in a diver-collected sample from Strangford Lough measured 160 mm and was aged at >10 years using scallop ring analysis. Given the large difference in shell size that exists in the scallop fishery, which is even larger in terms of the relative difference in body weight, the influence of scallop size must be considered as a factor that might contribute to variation in toxin concentrations both between samples and within samples.

Jones et al. (1992) reported higher weight-specific filtration rates in smaller mussels, *Mytilus edulis*, compared to larger ones. The occurrence of a similar difference in scallops in the field, assuming that different-sized scallops both filtered toxic cells, might contribute to faster rates of toxin accumulation in smaller individuals. Not only might differences occur in the rate of toxin uptake but also in the rate of toxin loss. The depuration rate of DA from small blue mussels, *Mytilus edulis*, has been recorded as being faster than in larger mussels (Novaczek et al. 1992). Variations in toxin concentration and shellfish size have also been reported in field studies with PSP toxins in the Mediterranean mussel, *Mytilus galloprovincialis* (L.) and with DSP toxins in *Mytilus edulis* (Morono et al. 2001, Strohmeier et al. 2005). Despite the data from both laboratory and field studies with mussels, investigations with king scallop have to date reported no evidence for a relationship between toxin concentration and scallop size, though these investigations only comprised data on one sampling occasion from two sites in Spain (Arévalo et al. 1998) and from one sampling occasion at a site in Scotland (Campbell et al. 2001).

In this research programme the relationship between scallop size and DA concentration was investigated on ten sampling occasions in samples from Clew Bay, County Mayo between February 2003 to February 2004 (Paper 1), on one sampling occasion at five



sites in the southeast coast scallop fishery (Paper 2), on two sampling occasions at a site in Strangford Lough, County Down (unpublished) and on three sampling occasions at six sites around the Isle of Man (Paper 5). Individual analysis of the hepatopancreas of each scallop was undertaken to provide data on inter-animal variability within samples from different locations. A summary of the results is provided in Table 3. Overall the relationship between DA concentration in hepatopancreas and scallop shell length exhibited both significant negative correlations, the absence of any correlation and significant positive correlations.

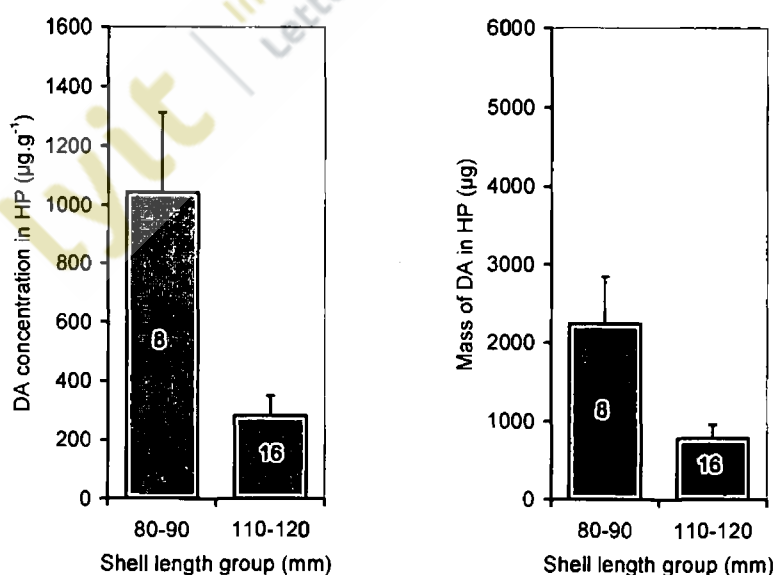
In the Clew Bay study a wide range of scallop shell lengths were analysed, as suggested by Campbell et al. (2001). A negative correlation between DA concentration and scallop shell length ( $R^2 = 0.7909$ ,  $n = 34$ ) was exhibited on the first sampling occasion (26<sup>th</sup> February 2003) though scallops in only three size groups (small, medium and large) were provided for analysis on this date. Mean toxin concentration in hepatopancreas of small scallops was  $1066.8 \pm 242.5 \mu\text{g.g}^{-1}$ , in medium-sized scallops  $323.2 \pm 68.6 \mu\text{g.g}^{-1}$  and large-sized scallops  $272.6 \pm 59.8 \mu\text{g.g}^{-1}$  (Paper 1). Negative correlations between toxin concentration and shell size have been reported in other bivalve species. Smaller mussels, *Mytilus edulis*, of 3-4 cm length accumulated relatively more mytilotoxin than larger mussels greater than 6cm length (Aalivk et al. 1981). Similarly smaller mussels of 45-55mm length accumulated more DA toxin than larger mussels of 60-70mm length (Novaczek et al. 1992). A negative correlation between the concentration of PSP toxins and shell size was also reported in Mediterranean mussels, *Mytilus galloprovincialis*, (Moroño et al. 2001) and in surf clams, *Spisula solidissima*, (Bricelj et al. unpublished data, in Bricelj & Shumway, 1998). In a field study the concentration of DSP toxin was inversely related to the meat content of blue mussels, *Mytilus edulis*, (Strohmeier et al. 2005). In this latter field study it was suggested that the negative correlation between toxin concentration and shellfish size might be due to faster depuration rates and consequently lower toxin content in larger-sized blue mussels. The negative correlation observed in the Clew Bay study was recorded in the first sample provided and hence it was impossible to confirm whether the scallops were accumulating or depurating toxin at the time of sample collection (Paper 1).



Sampling location	Date	N	DA concentration in hepatopancreas				Shell length range (mm)			R <sup>2</sup>	Reference
			Mean ± St. Dev. (µg g <sup>-1</sup> )	C.V. (%)	Min (µg g <sup>-1</sup> )	Max (µg g <sup>-1</sup> )					
Clew Bay											
	26-Feb-03	34	524.0 ± 381.7	72.8	108.4	1348.1	78	-	117	0.71	Paper 1, 5
	03-Apr-03	46	520.4 ± 254.2	48.8	16.6	1235.5	75	-	124	0.20	
	23-May-03	48	465.8 ± 127.8	27.4	136.5	715.0	80	-	120	0.08	
	03-Jul-03	46	166.8 ± 89.7	53.8	42.0	486.6	76	-	125	0.02	
	30-Jul-03	47	320.3 ± 95.7	29.9	50.3	590.4	67	-	132	0.12	
	03-Sep-03	48	247.6 ± 77.5	31.3	61.2	411.3	80	-	126	0.05	
	25-Sep-03	48	224.2 ± 69.5	31.0	95.3	422.2	80	-	135	0.01	
	12-Nov-03	48	209.0 ± 118.6	56.8	25.7	419.0	58	-	121	0.45	
	04-Dec-03	48	192.7 ± 109.7	56.9	22.0	530.4	69	-	124	0.35	
	11-Feb-04	46	167.7 ± 86.8	51.8	33.3	348.5	74	-	130	0.47	
Mine Head/Saltees grounds											
Station 5	17-Feb-04	48	422.3 ± 220.7	52.3	38.8	913.4	75	-	130	0.22	Paper 2, 5
Station 36	18-Feb-04	37	310.5 ± 134.0	43.2	95.7	695.8	75	-	130	0.19	
Station 44	20-Feb-04	48	262.5 ± 133.5	50.8	42.4	586.1	76	-	125	0.29	
Station 70	20-Feb-04	44	40.4 ± 19.0	47.0	14.0	90.7	65	-	125	0.00	
Station 94	24-Feb-04	40	242.9 ± 86.6	35.7	74.9	528.3	73	-	131	0.01	
Isle of Man grounds											
Targets	11-Nov-03	12	207.0 ± 63.6	30.7	107.6	325.5	100	-	144	0.20	Paper 4, 5
Peel	11-Nov-03	12	250.9 ± 129.5	51.6	124.1	558.6	75	-	140	0.48	
Bradda Inshore	11-Nov-03	11	191.7 ± 134.2	70.0	33.4	404.0	78	-	125	0.68	
Bradda Offshore	11-Nov-03	12	296.3 ± 104.5	35.3	156.3	498.9	102	-	126	0.54	
East Douglas	11-Nov-03	10	1.6 ± 1.6	97.5	0.0	4.8	102	-	142	0.10	
Laxey	11-Nov-03	12	0.2 ± 0.5	202.4	0.0	1.5	85	-	119	0.07	
Strangford Lough											
	18-Jun-03	46	1449.8 ± 350.9	24.2	279.6	1941.8	60	-	160	0.08	Paper 5
	01-Sep-03	48	2697.0 ± 591.7	21.9	1258.4	3834.4	52	-	152	0.29	
Kilkieran Bay											
	02-Jul-03	26	40.8 ± 14.0	34.3	22.6	78.2	93	-	159	0.03	Paper 5

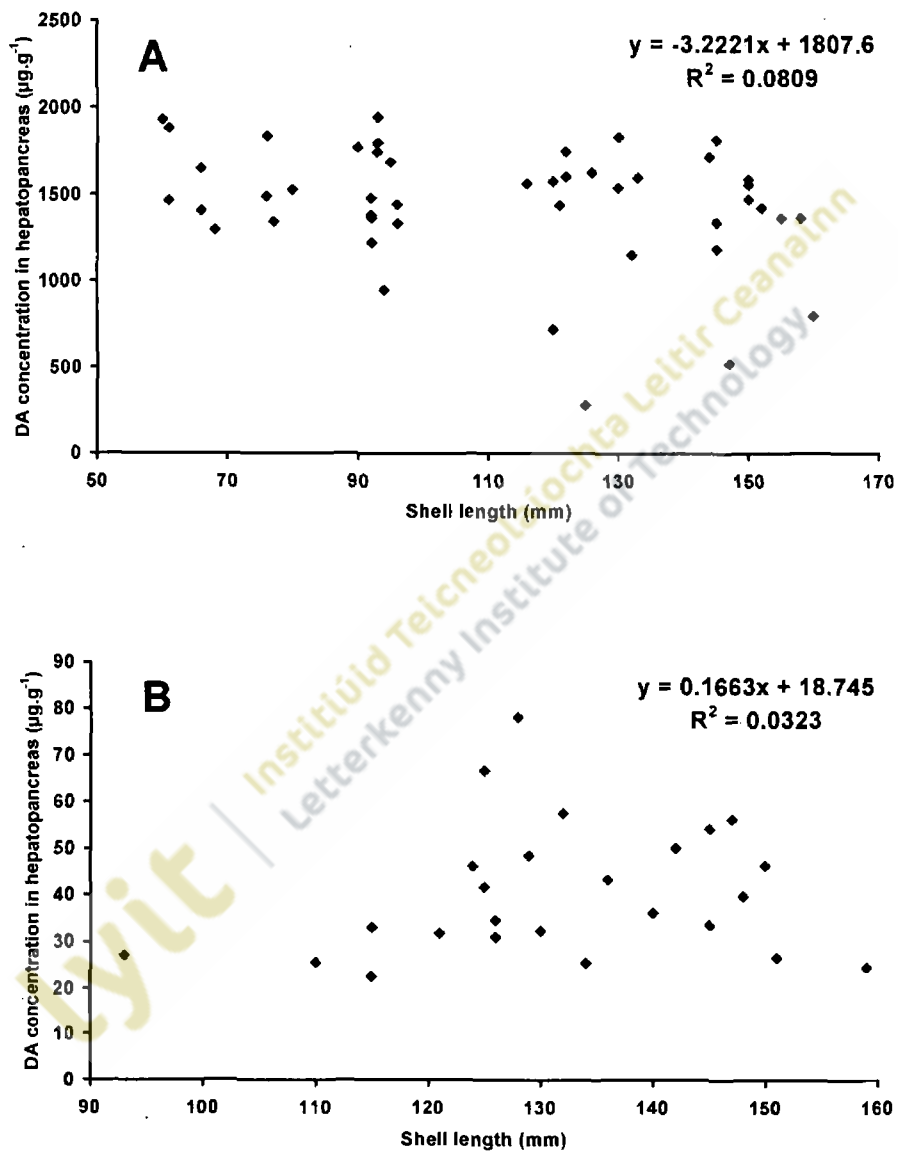
**Table 3.** Review of relationship (R<sup>2</sup>) of DA concentration in hepatopancreas with scallop shell length from Clew Bay (Paper 1, 5), the Mine Head/Saltees grounds (Paper 2, 5), the Isle of Man grounds (Paper 4, 5), Strangford Lough (Paper 5) and Kilkieran Bay (Paper 5).

On the second sampling occasion in the Clew Bay study, increases in DA concentration in the hepatopancreas of medium, large and very large scallops were recorded but no increase in the toxin concentration of small scallops, suggesting that overall scallops were in a toxin accumulation period. Based on these two datasets, it was suggested that the higher DA concentrations in the hepatopancreas of smaller scallops in the first sample might have resulted from higher rates of DA uptake (Jones et al. 1992) or from a higher assimilation rate in smaller scallops (Morono et al. 2001). Alternatively, the differences in DA concentration between smaller and larger scallops may have resulted from differences in the weight of the hepatopancreas between the size groups. If the rate of DA uptake between different sizes of scallops were similar then differences in hepatopancreas weight could result in different levels of toxin dilution. In such circumstances, small-sized scallops having a smaller hepatopancreas would have reduced toxin dilution and hence a higher toxin concentration. Consideration of the mass of DA in the hepatopancreas of both small and larger scallops from the first Clew Bay sample demonstrated that small scallops had not only a higher concentration of DA, but also a higher mass of DA. This supported the hypothesis that differences in DA concentration were caused by a faster rate of DA uptake or assimilation in smaller scallops, though a dilution effect could not be entirely discounted (Figure 9).



**Figure 9.** DA concentration and DA mass in the hepatopancreas of small (left) and large (right) scallops from Clew Bay, Co. Mayo on 26<sup>th</sup> February 2003. Sample number indicated in each bar.

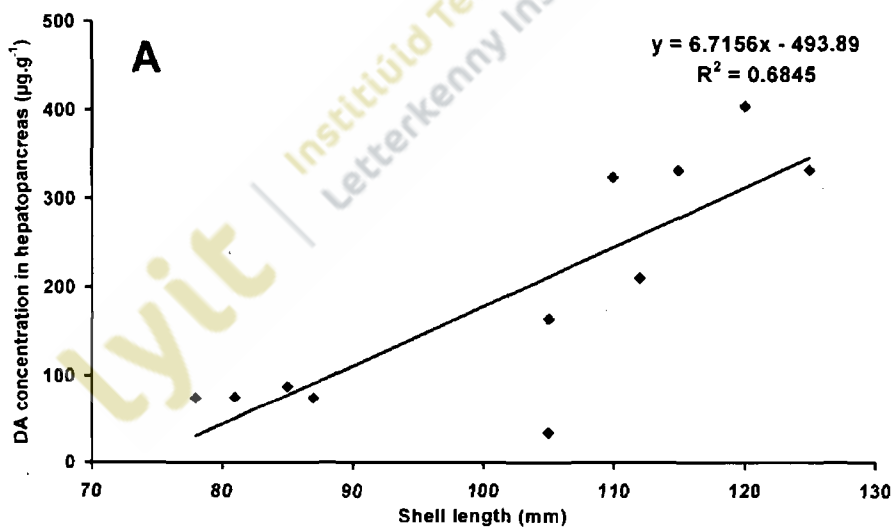
No correlation between DA concentration in the hepatopancreas and scallop shell length was exhibited on sixteen different sampling occasions (Table 3). Examples of the absence of a relationship are provided below in Figure 10.



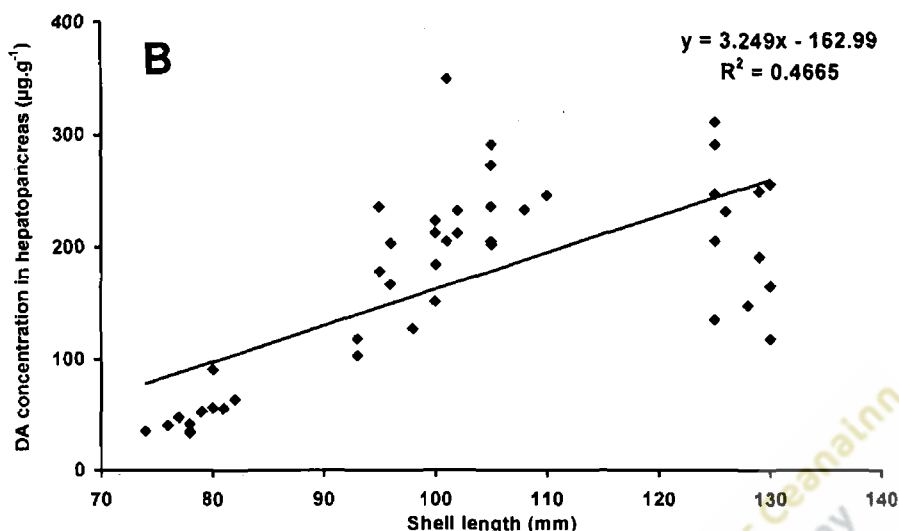
**Figure 10.** No correlation between DA concentrations in hepatopancreas and scallop shell lengths from Strangford Lough, County Down (A) and Kilkieran Bay, County Galway (B).

Absence of a correlation between DA concentration and the size of king scallop, *Pecten maximus*, have been reported from Ria de Arousa ( $R^2 = 0.040$ ,  $n = 72$ ) and from the Rias of Muros-Noia, Arousa and Vigo ( $R^2 = 0.038$ ,  $n = 61$ ) (Arévalo et al. 1998). Data from this second set of results involved the combination of scallops from three geographically distinct sample locations in to one sample and is considered of limited value for comparative purposes. Similar absence of a correlation between DA concentration and the shell length of sand crabs, *Emerita analoga*, (Powell et al. 2002) and common cuttlefish, *Sepia officianalis*, (Costa et al. 2005) has been reported. In a study of the relationship between PSP toxin concentration and the weight of geoduck clams, *Panope abrupta*, no relationship was reported over a five-month period (Curtis et al. 2000).

Significant positive correlations and significant but weak correlations between DA concentration in hepatopancreas and scallop shell length were reported on six sampling occasions. Examples of positive correlations are provided in Figure 11.



**Figure 11.** Positive correlation between DA concentrations in hepatopancreas and scallop shell length from Bradda Inshore, Isle of Man.



**Figure 11.** Positive correlation between DA concentrations in hepatopancreas and scallop shell length from Clew Bay on 11<sup>th</sup> February 2004.

To understand the cause of the positive correlations, the concentration of DA in the hepatopancreas of small (80-90 mm) and large (110-120 mm) scallops from Clew Bay on 25<sup>th</sup> September 2003 when no correlation ( $R^2 = 0.01$ ) was exhibited and 12<sup>th</sup> November 2003 when a positive correlation was exhibited ( $R^2 = 0.45$ ) were compared. Mean DA concentration in small scallops exhibited a significant decline from  $252.7 \pm 80.4 \mu\text{g.g}^{-1}$  to  $101.3 \pm 45.6 \mu\text{g.g}^{-1}$  compared to an increase exhibited by larger scallops from  $221.8 \pm 57.8 \mu\text{g.g}^{-1}$  to  $283.7 \pm 103.7 \mu\text{g.g}^{-1}$ . By 11<sup>th</sup> February 2004, the last sampling date in the Clew Bay study (Table 2), when again a positive correlation was exhibited, DA concentration in small scallops had declined further to  $66.0 \pm 16.4 \mu\text{g.g}^{-1}$  whilst that of the one scallop in the large size group was  $245.3 \mu\text{g.g}^{-1}$ . The most probable explanation for the positive correlations between DA concentration and scallop shell length is suggested to be faster depuration rates in smaller-sized scallops. In agreement with the proposed hypothesis to explain positive correlations a study by Novaczek et al. (1992) reported that blue mussels, *Mytilus edulis*, had faster rates of DA depuration in smaller mussels (45-55mm) compared to larger mussels (60-70mm). According to Novaczek et al. (1992) smaller mussels carried more DA toxin per unit body weight when

compared with the larger mussels and depurated more quickly than larger mussels. These findings were consistent with those from earlier laboratory studies with small and large mussels (Schulz-Baldes 1974, Widdows et al. 1979, Hawkins et al. 1990).

Mean DA concentration in hepatopancreas exhibited similar highly significant negative and positive correlations with both total tissue weight and hepatopancreas weight. Correlation coefficients were generally slightly lower than those between DA concentration in hepatopancreas and shell length except for one sample from the Peel fishing ground in the Isle of Man where a higher positive correlation was exhibited between DA concentration in hepatopancreas and total tissue weight (Paper 4, 5).

Results in which no correlation between DA concentration in hepatopancreas and scallop size were reported, excluding the two low concentration results from the Isle of Man (Paper 4, 5), exhibited reduced inter-animal variation (CV) compared to results in which negative or positive correlations between toxin concentration and shell length were reported. There was a very significant correlation between the CV's of DA concentration in hepatopancreas and  $R^2$  from the relationship between shell length and DA concentration in hepatopancreas ( $F_{1,20} = 14.91$ ,  $p = 0.001$ ,  $R^2 = 0.43$ ). This suggested that during periods of change in DA concentration, when inter-animal variability increased, i.e. during periods of toxin accumulation or depuration, the correlation between toxin concentration and shell length also increased (Paper 5).

The relationships between DA concentration in composite samples of gonad and scallop size and that between composite samples of adductor muscle and scallop size were both investigated (Paper 1, 2, 3, 4). In contrast to the individual analysis of each hepatopancreas, composite samples of each of these tissues were analysed since preliminary studies suggested that DA concentration in these tissues would be much lower than in hepatopancreas and hence if differences occurred would be more difficult to confirm due to the lower concentrations. Lower DA concentrations in gonad tissue compared to hepatopancreas (Paper 1, 2, 3, 4) and the high variability in toxin concentration associated with this organ (Arévalo et al. 1998, Campbell et al. 2001,

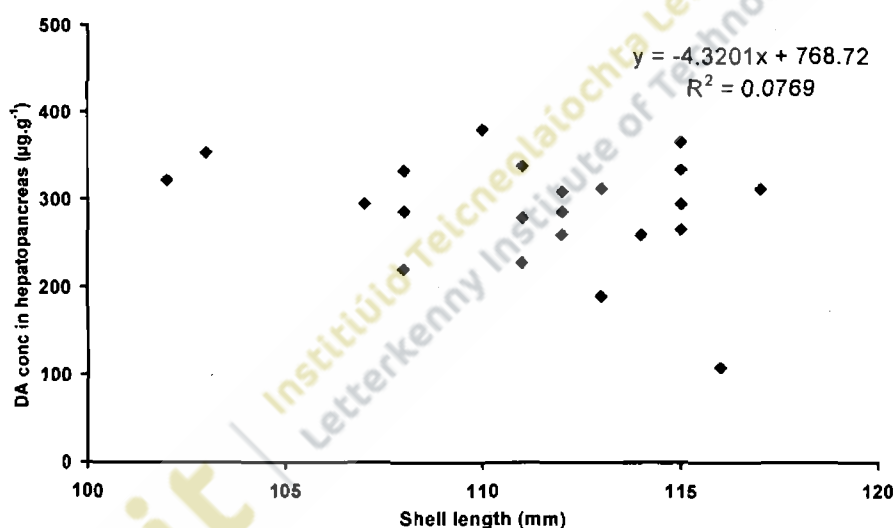
Paper 2) presented difficulties in understanding the relationship between DA toxin concentration and scallop size. The temporal studies in Clew Bay (Paper 1, 3) provided evidence of a relationship between DA concentration in gonad and gonad weight demonstrating the dilution effect of this organ on toxin concentration. To overcome the dilution effect caused by changes in gonad mass due to the reproductive cycle, changes in the mass of DA toxin in gonad were also examined (Paper 1, 3). Changes in the mass of DA in gonad over the 12-month study period in Clew Bay exhibited a similar trend to those for both the concentration and the mass of DA in hepatopancreas (Paper 1, 3). Given these similarities, albeit at much lower concentrations, it has been assumed that similar hypotheses to those used to explain changes in toxin concentration in the hepatopancreas are valid. Because the concentrations of DA in adductor muscle were even lower than those in gonad no attempts were made to understand the relationship with scallop size.

Further planned research work to test the hypotheses used to explain the negative and positive correlations between the toxin concentration in hepatopancreas and scallop shell length by transferring scallops of a range of sizes from a site with low background DA concentration into a site in which a toxic *Pseudo-nitzschia* bloom was developing proved impossible due to insufficient advance warning of toxic bloom development.

In contrast to the findings of other studies with DA and king scallops, the results demonstrated that scallop shell length could possibly influence DA toxin concentration (Arévalo et al. 1998, Campbell et al. 2001, Paper 1, 2, 4, 5). Smaller scallops exhibited higher DA concentrations in the hepatopancreas during toxin accumulation and lower DA content in the hepatopancreas during toxin depuration resulting in negative and positive correlations between DA concentration in scallop hepatopancreas and shell length respectively. From a regulatory perspective, the findings suggest that scallop shell length should be considered during the design and implementation of sampling protocols for the monitoring of DA concentration in king scallop.



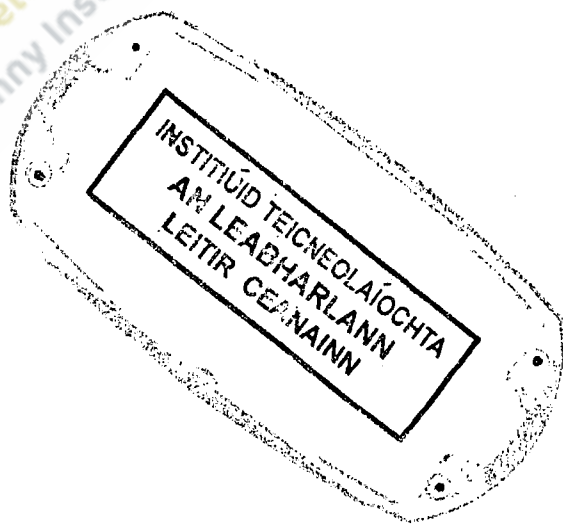
However, EU Commission Decision 2002/226/EC makes reference to the testing of ‘harvestable’ product, implying that for the national monitoring programme king scallops should be above the legal landing size of 100 mm in accordance with EU regulations, Annex XII of 850/98. Hence to assess whether scallop size should be considered in the design of a sampling protocol for a national monitoring programme those datasets that exhibited either a positive or negative correlation were re-examined, using only scallops greater than 100mm shell length. Data from Clew Bay on the first sampled occasion, 26<sup>th</sup> February 2003 ( $R^2 = 0.7079$ ) presenting a negative correlation with the complete dataset (Paper 1), exhibited no correlation with shell length when scallops less than 100 mm size were excluded (Figure 12).



**Figure 12.** Absence of a correlation between DA concentration in hepatopancreas and shell length of king scallop greater than 100mm from Clew Bay on 26<sup>th</sup> February 2003.

Of the six positive correlations reported previously (Paper 1, 4), only that from Bradda Offshore, Isle of Man sampled on 11<sup>th</sup> November 2003, which comprised twelve scallops greater than 100 mm shell length, exhibited a positive correlation when re-examined.

EU Commission Decision 2002/226/EC also makes reference to the testing of ‘edible parts’. Given that these edible parts, the adductor muscle and the gonad in this instance, have much lower DA concentrations than those in the hepatopancreas and that even using the DA concentration in the hepatopancreas only one positive correlation remained with commercial-sized scallops, it is considered unlikely that any significant correlation could be determined between DA toxin concentration in the edible parts and scallop size. As a consequence it is concluded that any changes to the current sampling protocols to take account of scallop size would not be warranted.



#### 4. Variability in DA concentration with water depth

Significant variability in the concentration of DA in king scallops has been reported in the field. Many physical and biological factors including water depth have been suggested as influencing toxin concentration in this bivalve species. In both national monitoring programmes and EU regulations there are no references to the water depth from which scallops are to be procured for monitoring purposes. Various relationships between toxin concentration and water depth have been reported in the literature (Cembella et al. 1994, Curtis et al. 2000, FSA 2001, Haya et al. 2003, Garcia et al. 2004).

In this research programme the relationship between DA concentration in king scallop, *Pecten maximus*, and water depth was investigated in the fishery off the southeast coast of Ireland on two sampling occasions (Paper 2, 6), in the Isle of Man fishery on three sampling occasions (Paper 4) and at a site in Strangford Lough, County Down on one sampling occasion (unpublished). For comparative purposes, data from an undergraduate study of variation in DA concentration with water depth in king scallop from Beirtrabui Bay, County Galway have been included (Bogan 2002). Though not directly comparable with the above datasets, some results from a temporal study investigating DA concentration in scallop from both the seabed and in suspension culture at one location in Clew Bay, County Mayo from February 2003 to February 2004 have also been included for discussion (Paper 3). Over the duration of these studies, DA concentration in hepatopancreas ranged from  $2158 \mu\text{g}\cdot\text{g}^{-1}$  in Strangford Lough (unpublished) to below the LOQ by HPLC ( $1.0 \mu\text{g}\cdot\text{g}^{-1}$ ) in the Isle of Man fishery (Paper 4). Inter-animal variation in DA concentration within a sample collected from the same sampling location and water depth was of a similar magnitude to values reported previously.

In the first study performed off the southeast coast of Ireland in September/October 2003, DA concentrations in king scallop from 69 sampling sites ranging in depth from 43 m to 75m were determined. All sampling sites were located within an area from  $51.55^\circ \text{N}$  to  $52.05^\circ \text{N}$  and  $6.60^\circ \text{W}$  to  $7.45^\circ \text{W}$ , less than the size of an ICES fishing box ( $0.5^\circ$  latitude by  $1^\circ$  longitude)(Paper 2).

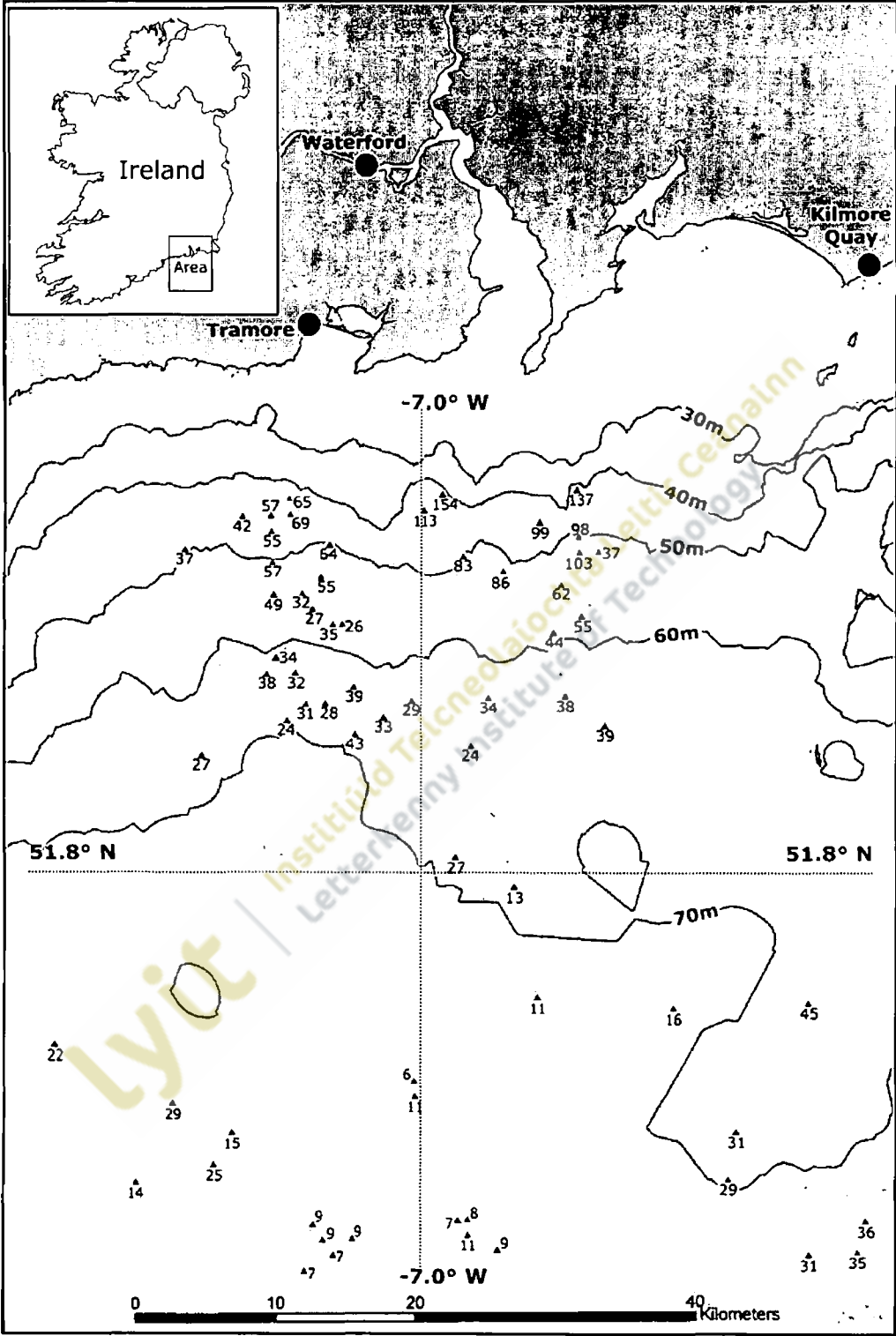
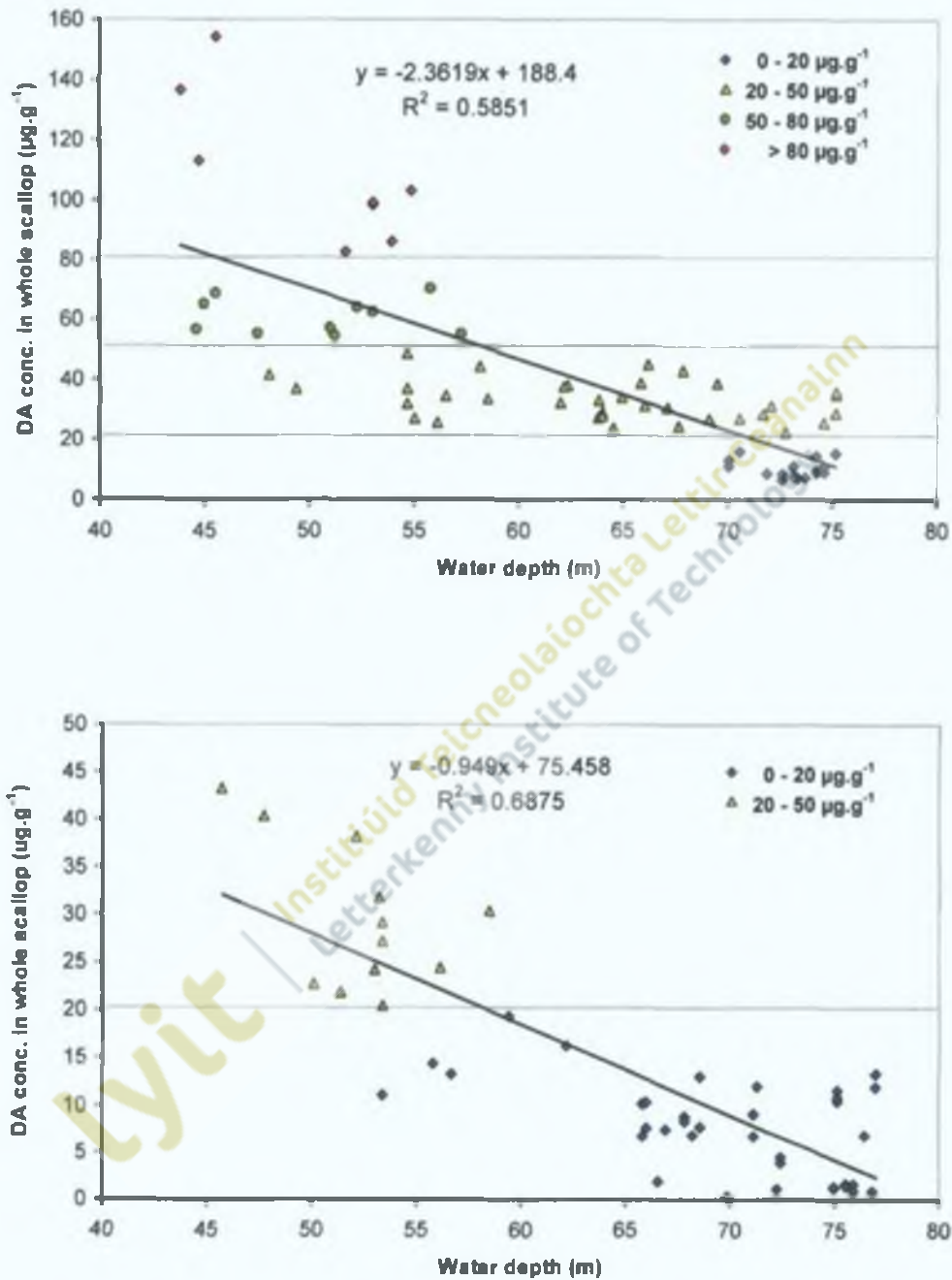


Figure 13. DA concentration ( $\mu\text{g.g}^{-1}$ ) in whole scallop *Pecten maximus* at 69 sampling stations off the southeast coast of Ireland.

Scallops from the 69 stations had similar mean physical characteristics, viz, length, height and age. Mean DA concentrations in whole scallop tissue ranged from  $6.5 \mu\text{g.g}^{-1}$  to  $154.3 \mu\text{g.g}^{-1}$ , with an overall mean of  $40.6 \pm 30.8 \mu\text{g.g}^{-1}$ . DA concentration in whole scallop exhibited a significant negative correlation with water depth ( $F_{1,67} = 94.26$ ,  $p = 0.000$ ,  $r = -0.765$ ) (Figure 14). Similar negative correlations between water depth and DA concentration in hepatopancreas ( $F_{1,67} = 71.98$ ,  $p = 0.000$ ,  $r = -0.72$ ), adductor muscle ( $F_{1,67} = 47.96$ ,  $p = 0.000$ ,  $r = -0.65$ ), and tissue remainder ( $F_{1,67} = 66.32$ ,  $p = 0.000$ ,  $r = -0.71$ ) were recorded (Paper 2). Shallower sampling stations in which higher DA concentrations occurred were closer inshore in this study and directly opposite Waterford Harbour. The possibility cannot be ignored that high DA concentrations may in part have resulted from geographical position rather than from water depth alone (Paper II). There was low correlation between DA concentration in gonad and water depth ( $F_{1,67} = 5.92$ ,  $p = 0.018$ ,  $r = -0.29$ ) attributed to high variability in the toxin concentration in this tissue which reflects a combination of the amount of DA in the intestinal loop and the mass of gonad tissue present at a particular stage of the reproductive cycle (Paper 2). Similar difficulties in understanding trends in toxin concentration in scallop gonad samples have been widely reported in the literature (Arévalo et al. 1998, Hess et al. 2001, Campbell et al. 2001, 2003, Fryer et al. 2002, Paper 1, 2, 3).

A second study in the same area off the southeast coast of Ireland was performed in September/October 2004 to determine if the negative correlation with water depth was transient or a consistent feature of this location. DA concentrations in whole tissue homogenates of king scallop tissues from 50 stations ranging in depth from 45 m to 77 m were determined. Concentrations were significantly lower than in the first study and ranged from  $43.24 \mu\text{g.g}^{-1}$  to below the LOQ by HPLC (Paper VI). Similar to the first study, DA concentration exhibited a significant negative correlation with water depth ( $F_{1,48} = 105.58$ ,  $p = 0.000$ ,  $r = -0.83$ ) (Figure 14). Similar negative correlations with  $R^2$  values of 0.47 and 0.70 between DA concentration in whole tissue homogenates of king scallop and water depths up to 80 m were also reported in Scottish waters on two sampling occasions in December and February 2001 (FSA 2001).



**Figure 14.** Relationship between DA concentration in tissue homogenates of whole scallop from the southeast coast of Ireland fishery and water depth in Sept/Oct 2003 (top) and Sept/Oct 2004 (bottom).

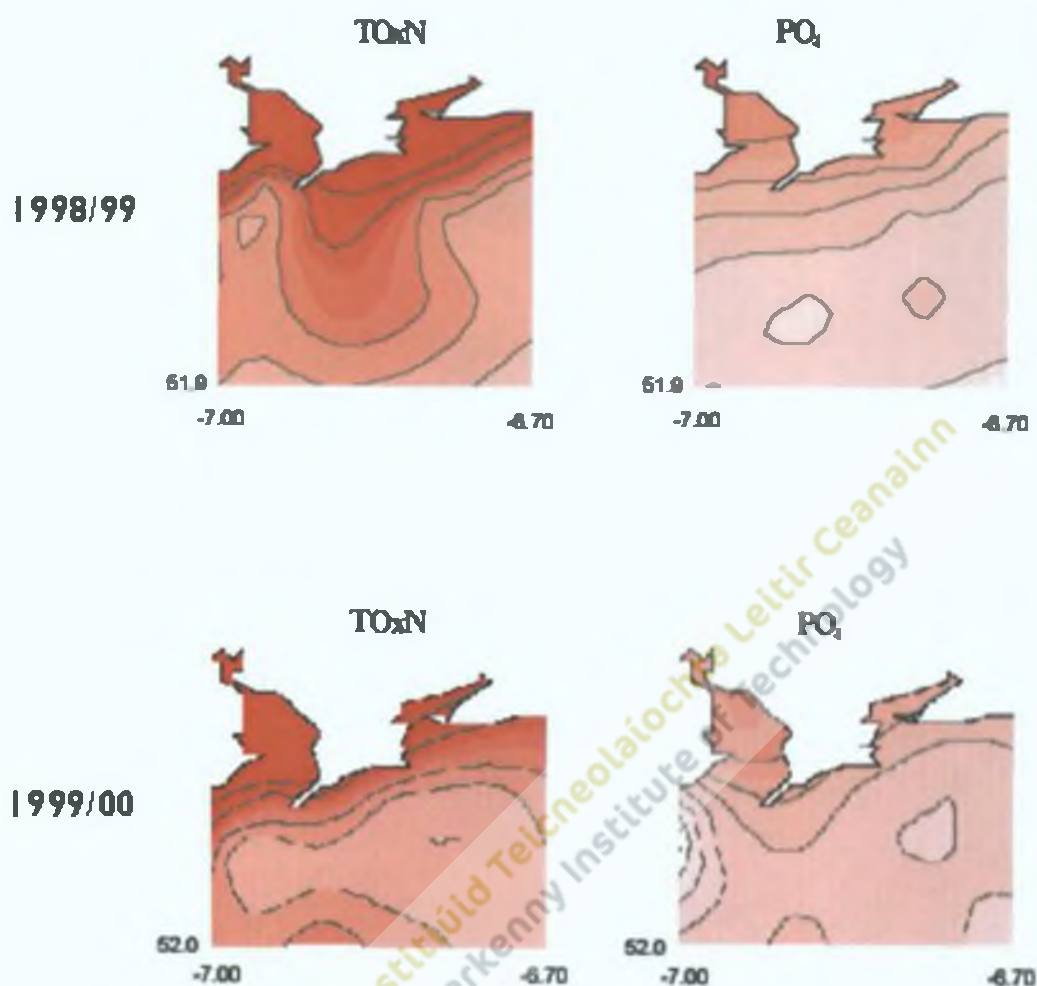


To understand the increased concentrations of DA in shallower sites, the origins of the toxicity and the factors that might have an influence firstly on its occurrence and secondly on its distribution have been considered.

Fresh water inputs carrying nutrients from adjacent rivers have been cited in many locations as a contributory factor to the formation of toxic blooms of the diatom *Pseudo-nitzschia* (Dortch et al. 1997, Bates et al. 1998, 2000, Trainer et al. 1998, 2000a, 2000b). In the plume of the Mississippi River in the northern Gulf of Mexico, high concentrations of *Pseudo-nitzschia* were recorded in sediment cores and associated with high river flow and nutrient inputs (Parsons et al. 2002). In both of the investigations off the southeast coast of Ireland, at sampling stations above 51.80° N, increased concentrations of DA were reported in scallops towards the coast, with the most elevated concentrations in the vicinity of Waterford Harbour (Bogan 2004a, Paper 2, 6). Three major rivers, the Nore, Suir and Barrow, with a combined total catchment area over 9,000 km<sup>2</sup>, flow into Waterford Harbour. Despite insignificant municipal inputs, annual riverine inputs of 19,640 tonnes total N and 766 tonnes total P have been estimated (Boelens et al. 1999). Elevated nutrient concentrations in the waters near Waterford Harbour following investigations performed over several winters have been reported (McGovern et al. 2002) (Figure 15).

A study of the fate of nutrients from the Waterford Harbour estuarine plume, reported that phytoplankton blooms were encouraged near the mouth of the estuary, in the region of nitrogen-rich freshwater and relatively phosphate-rich seawater (Raine et al. 2002). In summary, it is suggested that toxic *Pseudo-nitzschia* blooms could be promoted by nutrient-rich inflows into the waters close to Waterford Harbour, accumulated by filter feeding scallops, and result in elevated concentrations of DA. Similar nutrient enrichment in inshore waters caused by nutrient run-off from the land might explain the increased DA concentrations in scallops from the other inshore sampling stations.





**Figure 15.** Nutrient enrichment in the vicinity of Waterford Harbour in the winters of 1998/99 and 1999/2000 (from McGovern et al. 2002).

Differences in DA concentrations between inshore and offshore sites may also be influenced by the prevailing oceanographic conditions. Variations in food availability caused by currents and winds have been suggested as the cause of differences in the concentration of PSP toxin in geoduck clams, *Panope abrupta*, at various water depths (Curtis et al. 2000). Off the southeast coast, the stronger water currents prevailing in inshore waters have been suggested as the cause of faster scallop growth rates (Tully et al. 2002). Such currents may also be responsible for the provision of increased numbers of toxic *Pseudo-nitzschia* cells in inshore waters.

Differences in scallop toxicity between shallow and deep waters off the southeast coast may also be affected by the local oceanographic conditions in this area. The dominant hydrographic feature off the southeast coast of Ireland is the Celtic Sea Front, a tidal front that separates the waters of the Celtic Sea from those of the Irish Sea. These two water masses are quite distinct, the Celtic Sea exhibiting thermal stratification during the summer in contrast to the Irish Sea where tidally generated turbulence prevents the development of the seasonal thermocline. There is an array of *Pseudo-nitzschia* species in the thermally stratified waters on the Celtic Sea side of the front, but only two species in the mixed waters of the Irish Sea side have been reported (Cusack 2002). The physical barrier presented by the Celtic Sea Front could thus lead to bloom formation in some waters but not in others. Blooms of toxic *Pseudo-nitzschia* species formed offshore in one or other water mass may then be transported into other locations to induce molluscan intoxication. The locations into which such blooms are transported may well depend on the water mass within which they originated and local hydrographic conditions pertaining at the time. Similar transfers have been reported in waters off the west coast of the USA with offshore blooms proliferating in some parts but not others and their regional distribution being controlled by oceanographic conditions (Kellner, 2004). Both seasonal and geographical variability in the concentrations of PSP toxin in sea scallop, *Placopecten magellanicus*, between inshore (20 m depth) and offshore grounds (180 m depth) have been reported. In contrast to these studies high toxin concentrations were present throughout the year in offshore deeper water compared to lower toxin concentrations in early summer peaks in inshore areas. Variations in the levels of PSP toxin were attributed to geographical position rather than water depth alone (Cembella et al. 1993).

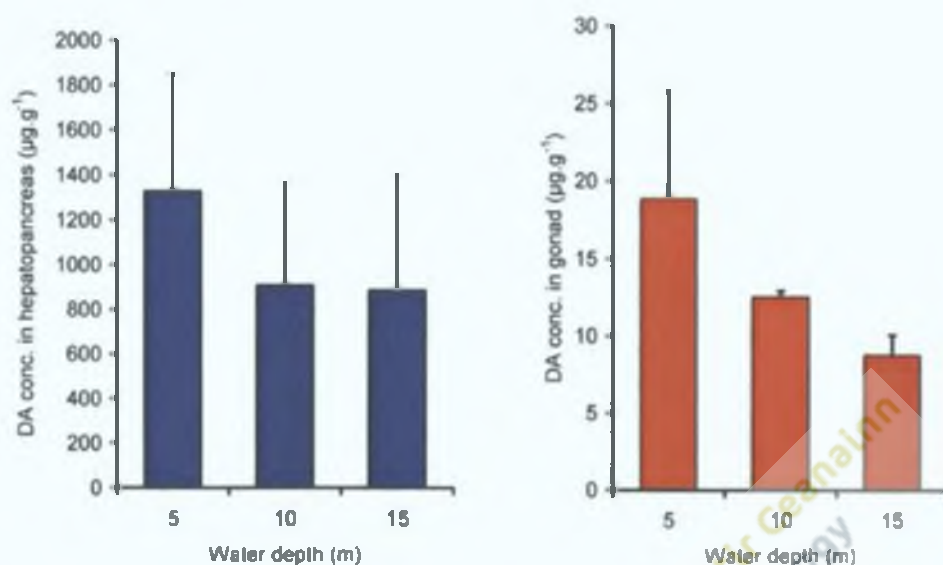
Commercial-sized king scallop collected by divers at 5m, 10m and 15 m from an aquaculture site in Marlfield Bay, Strangford Lough on 26<sup>th</sup> August 2004 also exhibited a negative correlation between DA concentration and water depth (unpublished data). Twelve scallops from each depth were collected and the DA concentration of each hepatopancreas determined to provide data on inter-animal variability at depth. DA

concentrations of composite gonad samples and composite adductor muscle samples from each depth were determined in triplicate. Results are provided in Table 4.

	Water depth (m)		
	5	10	15
Shell length (mm)	117.58 ± 5.01	115.50 ± 3.60	117.17 ± 5.13
Mean DA conc. in hepatopancreas (µg.g <sup>-1</sup> )	1327.42 ± 522.85	905.25 ± 459.60	884.71 ± 516.65
Mean DA conc. in gonad (µg.g <sup>-1</sup> )	18.86 ± 6.92	12.52 ± 0.40	8.77 ± 1.31
Mean DA conc. in adductor muscle (µg.g <sup>-1</sup> )	7.22 ± 6.35	Not detected	Not detected

**Table 4.** Mean shell length and mean DA concentration in tissues of king scallop collected from three different water depths in Strangford Lough, County Down.

King scallops from the different water depths were of similar shell length. DA concentration in hepatopancreas within samples exhibited high inter-animal variability, CV's ranging from 39.4 % to 58.4 %. Despite the declining trend in mean DA concentration in hepatopancreas with water depth, differences between toxin concentrations were not statistically significant, ( $n = 36$ ,  $F = 2.991$ ,  $p = 0.064$ ) (Figure 16). Mean DA concentrations in gonad followed the same trend as in the hepatopancreas, i.e. higher DA concentration in gonad was recorded in shallower water (Figure 16). Samples were only available on one occasion from this site in Strangford Lough and were limited to only three water depths. A similar trend in PSP toxin concentrations in geoduck clams, *Panope abrupta*, has been reported. Clams from deeper water depths (17 m) were consistently below the regulatory closure level and had considerably lower toxin concentrations than those in shallower waters (7 m) (Curtis et al. 2000).



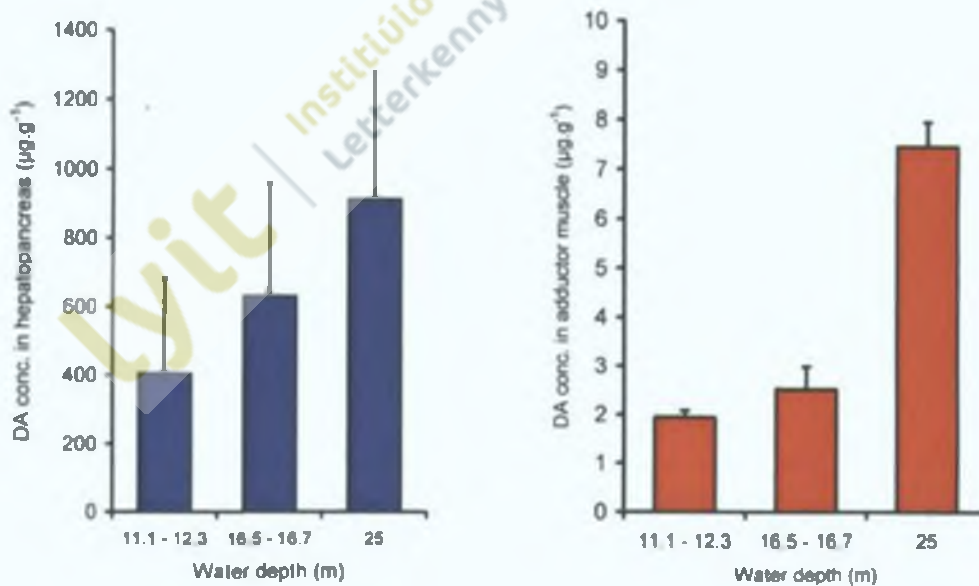
**Figure 16.** Variation in DA concentration in hepatopancreas and gonad of king scallop from Strangford Lough at three water depths. Error bars = 1 S.D.

In complete contrast to the results from the southeast scallop fishery and Strangford Lough, a significant positive correlation between mean DA concentration in hepatopancreas and water depth has been reported in scallops from Beirtrabui Bay, County Galway. Samples of twelve king scallop of approximately uniform size from three water depths: shallow (11.1-12.3 m), middle (16.5-16.7 m) and deep water (25 m) were collected for toxin analysis during a Bord Iascaigh Mhara (Irish Sea Fisheries Board) stock assessment survey (Bogan 2002). The DA concentration of each hepatopancreas was determined to provide data on inter-animal variability at depth. DA concentrations of composite gonad tissue and composite adductor muscle tissue from each depth were determined in triplicate. In this study samples after toxin extraction were cleaned-up using SAX cartridges prior to analysis using HPLC-UV. Results are provided in Table 5.

	Water depth range (m)		
	11.1 - 12.3	16.5 - 16.7	25
Shell length (mm)	128.40 $\pm$ 8.88	129.80 $\pm$ 7.24	132.80 $\pm$ 8.97
Mean DA conc. in hepatopancreas ( $\mu\text{g}\cdot\text{g}^{-1}$ )	404.30 $\pm$ 279.36	629.32 $\pm$ 327.99	911.85 $\pm$ 364.53
Mean DA conc. in gonad ( $\mu\text{g}\cdot\text{g}^{-1}$ )	2.86 $\pm$ 0.13	11.83 $\pm$ 0.15	6.94 $\pm$ 0.53
Mean DA conc. in adductor muscle ( $\mu\text{g}\cdot\text{g}^{-1}$ )	1.95 $\pm$ 0.14	2.52 $\pm$ 0.47	7.45 $\pm$ 0.51

**Table 5.** Mean shell length and mean DA concentration in tissues of king scallop dredged from three water depths in Bertrabui Bay, County Galway.

King scallops dredged from different water depths were of similar shell length. DA concentration in hepatopancreas within samples exhibited high inter-animal variability, CV's ranging from 40.0 % to 69.1 % and a positive correlation with water depth (Figure 17).

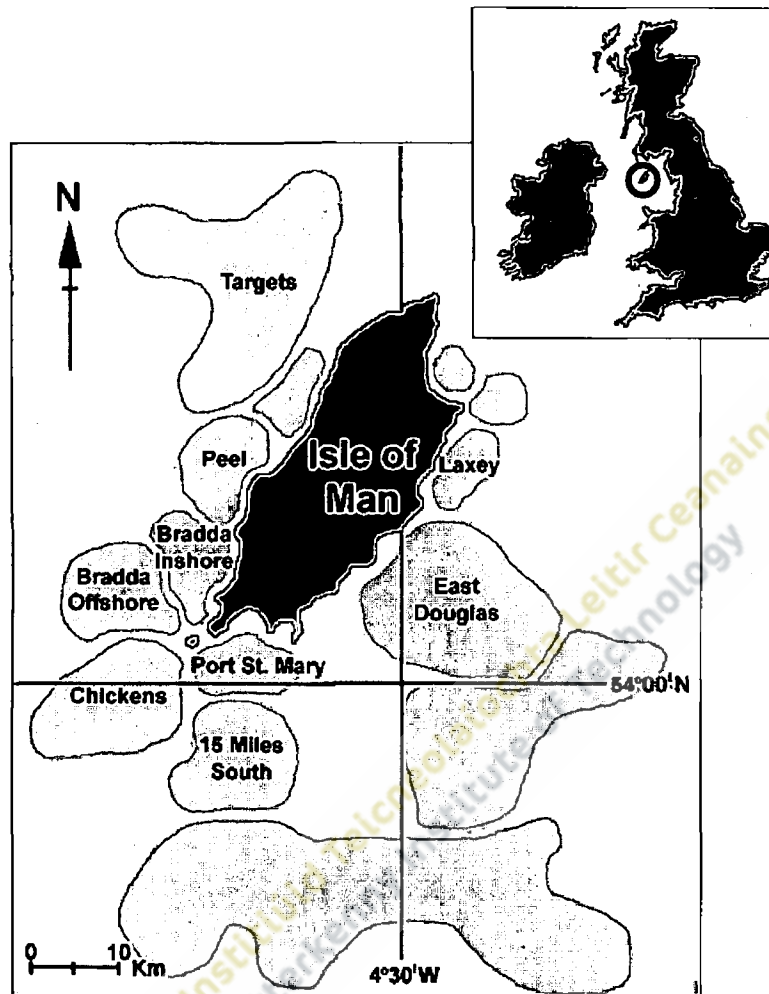


**Figure 17.** Variation in DA concentration in hepatopancreas and adductor muscle of king scallop from Bertrabui Bay at three water depths. Error bars = 1 S.D.

Statistical analysis using one-way ANOVA demonstrated that the mean DA concentrations in hepatopancreas were statistically different at the three depths, ( $n = 34$ ,  $F = 6.603$ ,  $p = 0.004$ ). Mean DA concentrations in adductor muscle followed the same trend as in the hepatopancreas, i.e. higher DA concentrations in adductor muscle were recorded in deeper water. Samples were only collected on one occasion from Beirtrabui Bay and were limited to only three water depths. Positive correlations between toxin concentration and water depth have also been reported elsewhere in the literature. In a two-year study of seasonal variations in PSP toxin concentrations in sea scallop, *Placopecten magellanicus*, from inshore (20 m) and offshore (180 m) waters were erratic, however during early summer of the second year, toxin concentrations were consistently higher in offshore waters (Cembella et al. 1994). Similarly mussels transferred into inshore locations exhibited PSP toxin concentrations two orders of magnitude lower than mussels suspended 300 m to 600 m offshore (Desbiens et al. 1990). These differences in toxin concentration with water depth may also be attributable to geographical location and the vertical distribution, persistence and magnitude of toxic phytoplankton blooms (Shumway and Cembella, 1993).

In addition to positive and negative correlations, some locations in this research programme exhibited no correlation between DA concentration and water depth. Such results were exemplified by three studies performed in scallop fishing grounds around the Isle of Man (Paper 4). A commercial fishery for king scallop has existed around the Isle of Man since 1937 (Beukers-Stewart et al. 2003). The fishery constitutes a significant natural resource with harvesting by boats from as far away as County Wexford in Ireland. Samples for DA analysis, each comprising twelve scallops, were dredged from the fishing grounds in mid-October 2003, mid-June 2004 and the end-October 2004 during stock assessment surveys. The mean depth from which samples were collected were as follows: 26.9 m (Laxey), 28.8 m (Targets), 33.4 m (Bradda Inshore), 34.1 m (Peel), 34.7 m (East Douglas), 41.4 m (Bradda Offshore), 63.6 m (Chickens) and 64.0 m (15-Miles South-Port St. Mary). Samples from Chickens and 15-Miles South-Port St. Mary were not available on the first sampling occasion in mid-October 2003. The location of the fishing grounds from which samples were collected is shown in Figure 18.

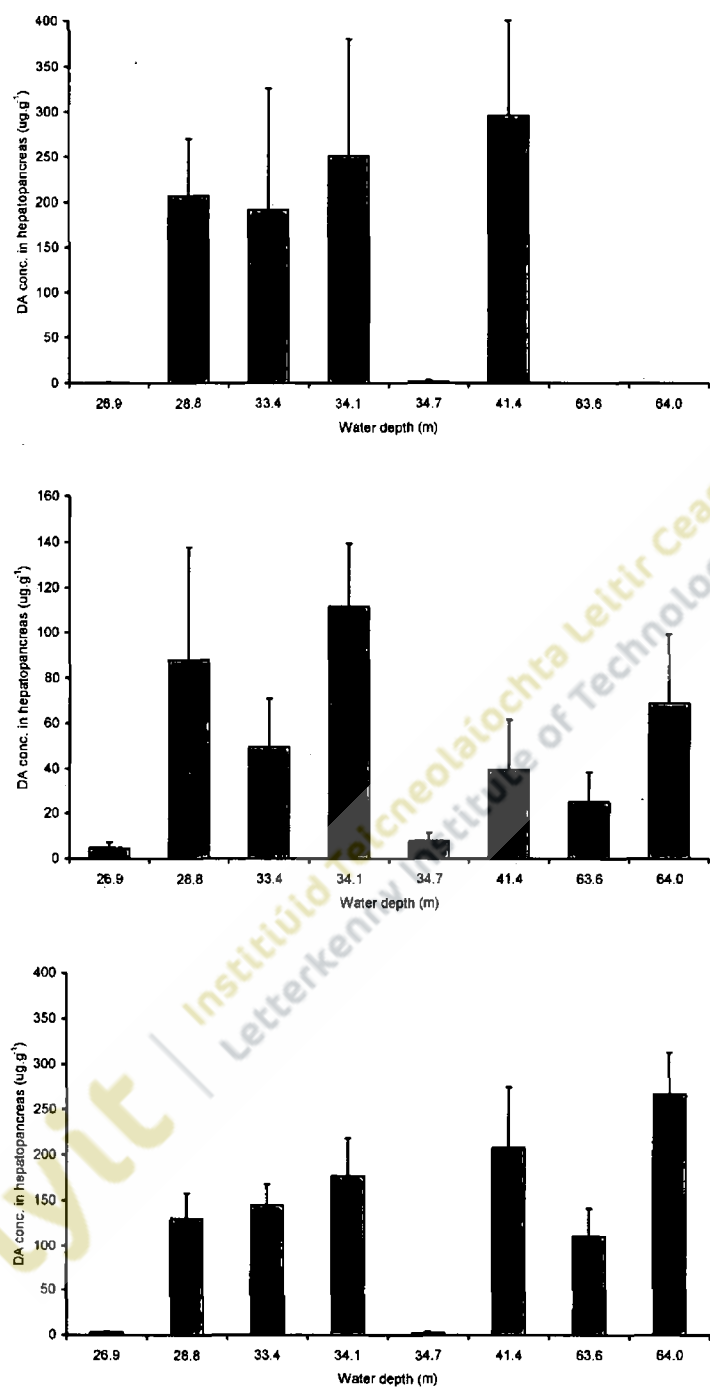




**Figure 18.** Map showing location of the main king scallop fishing grounds around the Isle of Man. Grounds sampled in this study are labelled in bold.

DA concentrations in hepatopancreas on the three sampled occasions exhibited no relationship with water depth (Figure 19). The most salient feature of the results from these studies was the significantly lower DA concentrations recorded from the two sites on the east coast of the Isle of Man, Laxey (26.9 m) and East Douglas (34.7 m) compared to the sites off the west coast of the Isle of Man. This trend in significantly lower levels of DA in scallops on the east coast was also reported by Shammon et al. 2004.





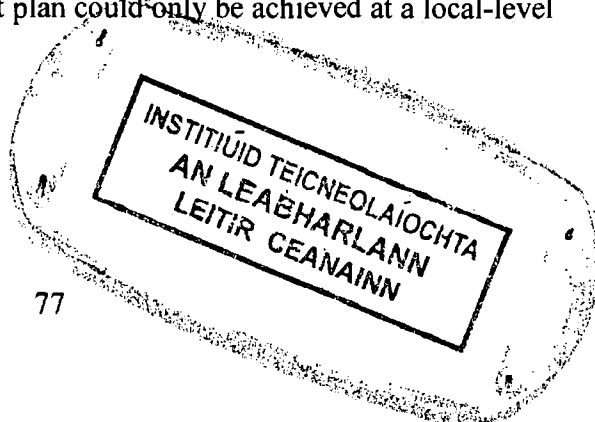
**Figure 19.** Relationship between DA concentration in hepatopancreas of king scallop from different water depths around the Isle of Man in October 2003 (top), June 2004 (middle) and October 2004 (bottom).

The clear difference in DA concentrations between scallops on the east and west of the Isle of Man suggested quite distinct oceanographic conditions on the two sides of the island. By comparison to the waters off the east of the Isle of Man, waters to the southwest are deeper with weak tidal currents allowing the seasonal development of stratification, which can become stable except in the area having a near-surface gyre driven by density gradients associated with cold bottom water (Slinn 1974, Hill et al. 1994, Gowen et al. 2005, Kennington et al. 2005). It has been suggested that the near-surface gyre in the southwest of the island coupled with elevated seawater temperatures recorded over the last decade may be responsible for the increased production of toxic phytoplankton in this area (Shammon et al. 2004). The DA concentrations in hepatopancreas of scallops from the east coast sites, the lowest concentrations in hepatopancreas recorded during this entire research programme (Paper 1, 2, 3, 4, 5, 6), suggest that an opportunity might exist to develop a market for fresh in-shell scallops from these sites. Such scallops command considerably higher prices than processed scallops and are highly sought after by top class restaurants, though further research with more regular sampling of scallops from the fishing grounds on the east coast of the Isle of Man would be required to confirm the results from these investigations.

Data suggesting the presence of correlations between toxin concentration and water depth are always liable to the claim that differences in the geographical location were responsible for the correlation since it is fundamentally impossible to have two water depths at one sampling site. A temporal study in Clew Bay, County Mayo performed between February 2003 and February 2004 was undertaken to monitor DA concentrations in scallops suspended from a submerged longline 2m beneath the water surface and scallops on the seabed (12-15m) immediately beneath the longline scallops (Paper 3). Although such an experimental design allowed scallops to be held at two different water depths at precisely the same geographical location, differences in the holding system were by necessity introduced. Mean DA concentration in hepatopancreas of scallops from the seabed and longline peaked in April 2003, individual concentrations reaching maxima of  $1037.1 \mu\text{g.g}^{-1}$  and  $1212.6 \mu\text{g.g}^{-1}$  respectively. No statistically significant differences were exhibited between DA concentration in hepatopancreas of seabed and

longline scallops except on one of the ten sampling occasions (Paper 3). Similarly no significant differences in PSP toxin concentration were demonstrated between sea scallop, *Placopecten magellanicus*, suspended 2 m beneath the water surface and scallops on the seabed at 11 m water depth (Haya et al. 2003). Interpretation of DA concentrations in gonad of scallops at the two water depths were complicated by the lower concentrations recorded and the variable size of the gonad due to the reproductive cycle over the 12-month study duration. DA concentrations in adductor muscle were below the limit of detection throughout the investigation (Paper 3). It has been suggested that a time delay in toxin accumulation between suspended and seabed scallops might be expected as cells from a toxic bloom descended to the seabed (Bricelj & Shumway, 1998). In a further study, longline mussels *Mytilus chilensis* were sampled at regular intervals along a drop-rope, from 1 m below the water surface down to 10 m depth and analysed for both DSP and PSP toxins. Lowest toxin concentrations were reported in mussels from the top and bottom of the drop-rope, highest toxin concentrations occurred at a depth of 6 metres (Garcia et al. 2004). On the basis of the toxicity results it was suggested that stratification of toxic dinoflagellates in the water column was responsible for peak toxicity at this depth (Garcia et al. 2004). In the Clew Bay study, since no difference in DA concentrations were reported between suspended culture and seabed scallops it might similarly be inferred that *Pseudo-nitzschia* cells at this site were widely dispersed throughout the water column.

In summary this research programme reported negative, positive and the absence of a correlation between DA concentration in tissues of the king scallop and water depth. The relationship appeared to be site specific since the correlation or lack of one was consistent whenever an investigation at a site was repeated. The results suggested that the relationship between toxicity and water depth might be a function of geographical location and hydrodynamics at a particular site. The incorporation of site-specific relationships into a biotoxin management plan could only be achieved at a local-level rather than at a national-level.



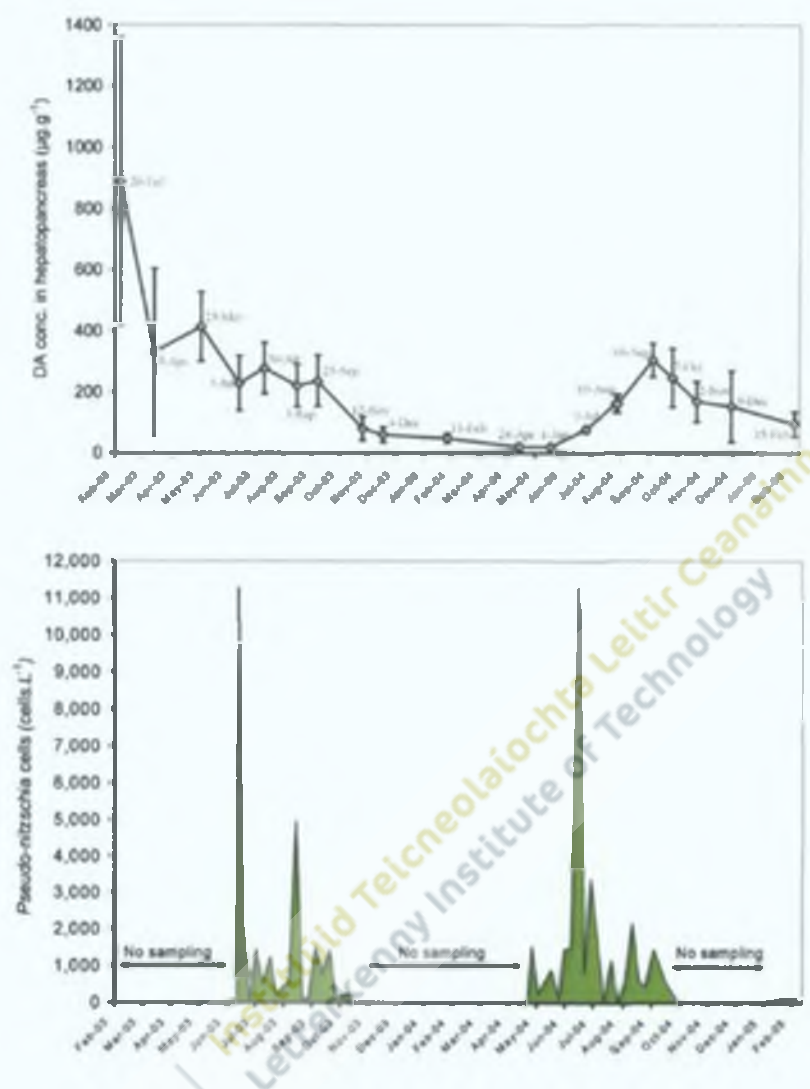
## 5. Understanding the causes of DA concentration variability

Significant differences in DA concentration between individual king scallop in a sample, manifested as high co-efficients of variation (CV), have been widely reported (Arévalo et al. 1998, Campbell et al. 2001, FSA 2001, Blanco et al. 2002, James et al. 2005, Paper 1, 2, 3, 4, 5, 6). Since the source of this toxin in many parts of the world has been established as toxic *Pseudo-nitzschia* species (Bates et al. 1989, Bates 2000, Hasle 2002, Cusack 2002, Fehling 2005) differences in DA concentration between individual scallop presumably arise through variations in the rate of filtering of these toxic cells from the water, variations in the rate of toxin sequestration and accumulation and/or variations in the rate of toxin depuration. To contribute to our understanding of the cause of variability several aspects associated with the uptake of DA toxin have been investigated in this research programme. These studies focussed on the correlation between toxin concentration and the occurrence of cells of *Pseudo-nitzschia* species, variations in toxin concentration at different positions in the water column and the possible uptake of DA toxin in the absence of toxic cells.

Several attempts to correlate the occurrence of DA toxin in bivalves and the abundance of cells of *Pseudo-nitzschia* species in the water have been reported. Although a moderate correlation was obtained between DA concentration in *Mytilus edulis* and the abundance of *Pseudo-nitzschia* cells in the water column, only a weak correlation was established in king scallop (FSA 2005). Dense blooms of *Pseudo-nitzschia* cells do not always coincide with high DA concentrations in bivalves (Dörtch et al. 1997, Parsons et al. 2002) and conversely no source in the water column could be determined to explain the high concentrations of DA in razor clams and Dungeness crabs from waters in the Pacific Northwest (Horner et al. 1997, Bates et al. 1998). In Irish waters, high concentrations of DA have been reported in king scallop samples despite the absence of significant numbers of cells of *Pseudo-nitzschia* (James et al. 2005).

In this research programme an investigation of the relationship between DA concentration in hepatopancreas of king scallop and the abundance of potentially toxic

*Pseudo-nitzschia* species was performed. Samples comprising twelve king scallops were diver-collected from a seabed aquaculture site in Clew Bay, County Mayo at approximately monthly intervals from February 2003 to February 2005. Since at the time of the investigation the effect of scallop size on toxin concentration had not been determined, scallop size for this study was standardised at 70-90 mm mean shell length (Paper 1). Subsequent results have demonstrated that the choice of small-sized scallops was a fortuitous one since smaller scallops exhibited faster rates of toxin accumulation and depuration (Paper 1, 5) and might therefore be expected to exhibit a better correlation with the abundance of toxic *Pseudo-nitzschia* cells in the water column. The procurement of samples by diver collection from a shallow-water aquaculture site (12 m to 15 m), rather than by dredging from offshore fishing grounds, ensured that variations in toxin concentration due to spatial distribution within the sampling area were minimised (Paper 2, 4). From a practical perspective the selection of a shallow inshore sampling site rather than an offshore site reduced the probability of restrictions in sampling frequency over the winter period given the 24-month study duration. The hepatopancreas of each scallop in the study was analysed individually to provide data on variability in DA concentration within each sample batch on each date. Composite samples of gonad and composite samples of adductor muscle from each sample batch were analysed because preliminary studies suggested that DA concentration in these tissues would be much lower than that in hepatopancreas and hence differences, if present, would be more difficult to confirm due to the lower concentrations present. Data on the abundance of cells of *Pseudo-nitzschia* species was obtained from the records of the National Phytoplankton Monitoring Programme of the Marine Institute (MI) and from the results of the MI-funded RTDI strategic research project on the biological oceanography of harmful algal blooms (BOHAB) undertaken by the National University of Ireland Galway. Phytoplankton samples were collected, when possible, on a weekly basis using a Lund tube from the sampling site “Clew Bay North”, designated as a sample collection point under the National Phytoplankton Monitoring Programme. The results of DA concentration in hepatopancreas and abundance of cells of *Pseudo-nitzschia* species over the period February 2003 to February 2005 are provided in Figure 20.



**Figure 20.** DA concentration in hepatopancreas of king scallop (top) and cell density of *Pseudo-nitzschia* species (bottom) from February 2003 to February 2005 in Clew Bay North. (Error bars = 1 SD).

DA concentrations in hepatopancreas of king scallop were at the highest level during this research investigation in the first sample collected in February 2003 and exhibited a mean concentration of  $889 \pm 469.6 \mu\text{g.g}^{-1}$ . Slow depuration occurred over the next 16-months to a concentration of  $21.1 \pm 4.4 \mu\text{g.g}^{-1}$  in June 2004. Coefficients of variation over this period ranged from 21.0 % to 82.5 %. From June 2004 to September 2004 mean DA concentration in hepatopancreas increased to  $306.8 \pm 54.5 \mu\text{g.g}^{-1}$  followed again by slow depuration until the date of the last sample in February 2005.



No phytoplankton data were available for the period from February 2003 to July 2003, somewhat unfortunate given the peak in DA toxicity in hepatopancreas recorded in February 2003. During 2003 the highest cell density of 11,280 cells.l<sup>-1</sup> was reported in the first phytoplankton sample on 2<sup>nd</sup> July. Sampling ceased in November 2003 and recommenced in March 2004. Highest cell density during 2004 was reported on 29<sup>th</sup> June at 11,280 cells.l<sup>-1</sup> again. Neither of these peaks in the abundance of cells of *Pseudo-nitzschia* species coincided with a peak in DA toxicity in scallop. Several smaller peaks in the range 1000 – 5000 cells.l<sup>-1</sup> were recorded during the 2003 and 2004 sampling seasons. None of these smaller peaks caused any increase in DA concentration in hepatopancreas during 2003. The increase in DA concentration in hepatopancreas over the period July 2004 to September 2004 could be associated with small peaks in the cell density of *Pseudo-nitzschia* species recorded on 12<sup>th</sup> July (3360 cells.l<sup>-1</sup>), 3<sup>rd</sup> August (1160 cells.l<sup>-1</sup>), 23<sup>rd</sup> August (2160 cells.l<sup>-1</sup>) and 15<sup>th</sup> September (1440 cells.l<sup>-1</sup>). However, since literature values for cell densities of *Pseudo-nitzschia* species during blooms are of the order of 100,000 cells.l<sup>-1</sup> it is suggested that these small peaks are of limited significance in the context of ASP toxicity (Bates et al. 1998).

The principle benefit advocated in support of phytoplankton monitoring is its potential to provide an early warning mechanism of impending toxicity. The results of this programme demonstrated that there is no correlation between the cell density of total *Pseudo-nitzschia* species and DA toxicity in scallops. Further work is required to examine the relationship between DA toxicity and the abundance of cells of the toxic *Pseudo-nitzschia* species present in Irish waters. Although the identification of toxigenic species in Irish waters using electron microscopy is possible (Cusack 2002), this technique is not considered a feasible option when rapid results are required to provide an early warning system of toxicity. A phytoplankton programme using molecular probes for the identification of toxic *Pseudo-nitzschia* species either by fluorescence *in situ* hybridisation (Miller et al. 1998, 2000), sandwich hybridisation techniques (Scholin et al. 1999, Ayers et al. 2005) or real-time PCR (Bowers et al. 2002) might offer potential as a technique for the provision of rapid data capable of providing the foundation for an early warning system.



Blooms of toxic *Pseudo-nitzschia* and other species are often regarded as being widely dispersed throughout the water column. In practise however, both vertical and horizontal gradients in the distribution of toxic cells exist which may lead to considerable patchiness in spatial distribution and, in turn, large variability in toxin concentrations in shellfish (Bricelj & Shumway 1998, MacKenzie et al. 2004, Fehling 2004, Townsend et al. 2005). Following the development of a bloom, toxic *Pseudo-nitzschia* cells have been reported to descend from nutrient-poor surface waters into higher-nutrient mixed layers and may contribute to differential profiles in toxicity at depth (Trainer et al. 1998, 2000). Toxic *Pseudo-nitzschia* cells at depth have been shown to remain toxic and it has been suggested that their upwelling may contribute to the seeding of a series of toxic pulses and hence persistence of shellfish toxicity (Dortch et al. 1997). Thin layers of *Pseudo-nitzschia* cells in the water column extending horizontally over large areas and at cell densities greater than  $10^6$  toxic cells.l<sup>1</sup> have been reported and may be responsible for unexplained toxicity events (Rines et al. 2002). As part of this research programme a comparative study of DA concentrations in scallop suspended from a submerged longline at 2 m water depth and scallop on the seabed at 12-15 m water depth was performed from February 2003 to February 2004 (Paper 3). No differences in toxin concentrations in hepatopancreas between scallops held in suspension or from the seabed were reported (Paper 3) suggesting that the source of DA toxicity was evenly distributed throughout the water column. Results from this site are in contrast to those reported with *Mytilus chilensis* at various depths from the water surface to 10 m in which the highest concentrations of PSP and DSP toxin were recorded at 6 m depth and attributed to different densities of toxic cells as a result of stratification (Garcia et al. 2004).

Recently a benthic pennate diatom *Nitzschia navis-varingica* isolated from a shrimp pond in Vietnam has been confirmed as a further source of DA toxin (Kotaki et al. 2000, Lundholm and Moestrup 2000, Kotaki et al. 2004). It has been suggested that this DA-producing benthic species may be widely distributed and contribute to variability of DA concentrations in bivalve species. This comparative study of DA concentration between scallops suspended at 2 m depth and scallops from the seabed provided no support for the presence of DA-producing benthic diatoms in this location (Paper 3).

Results from both field and laboratory studies have suggested that during bloom conditions significant amounts of toxin may be dissolved in seawater (Mackenzie et al. 1998, 2003). Following senescence of the bloom, algal cells may rupture or lyse releasing toxins into the water column (Steidinger and Baden 1983, Pierce et al. 2001). Blooming and/or rupture of toxic *Pseudo-nitzschia* cells may contribute to the release of a significant amount of DA into the water column and may explain DA concentrations observed in bivalves even in the absence of *Pseudo-nitzschia* cells. In this research programme a laboratory trial to investigate the uptake of dissolved DA in the absence of toxic *Pseudo-nitzschia* cells was undertaken in February 2004 using scallops of mean shell length  $107.5 \pm 6.7$  mm ( $n = 84$ ) and initial DA concentration in hepatopancreas of  $1.18 \pm 0.77$   $\mu\text{g.g}^{-1}$  (Paper 8). Scallops were randomly sub-divided into seven batches ( $n = 12$ ) and the DA concentration in individual hepatopancreas of one batch determined to establish the background toxin concentration. To investigate the possibility that non-toxic algal cells might serve as a vector for the transport of dissolved DA adsorbed onto non-toxic algal cells, the trial included treatments exposed to dissolved DA and either the diatom *Chaetoceros calcitrans* or the flagellate *Isochrysis galbana*. DA for the investigation was extracted from highly contaminated scallop hepatopancreas using methanol:water. Removal of methanol from the extract and concentration of the toxin in the extract was performed by rotary evaporation. Neither water exchange nor control of water temperature was used in this trial although water movement was effected using gentle aeration. The format of the laboratory trial is provided below in Figure 21.

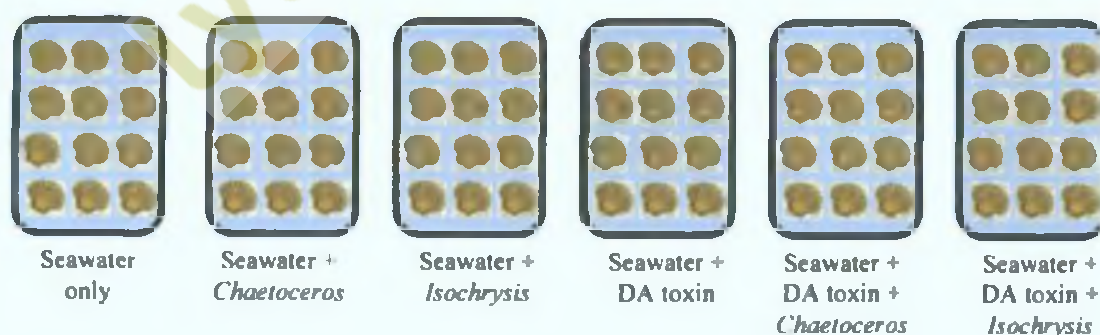
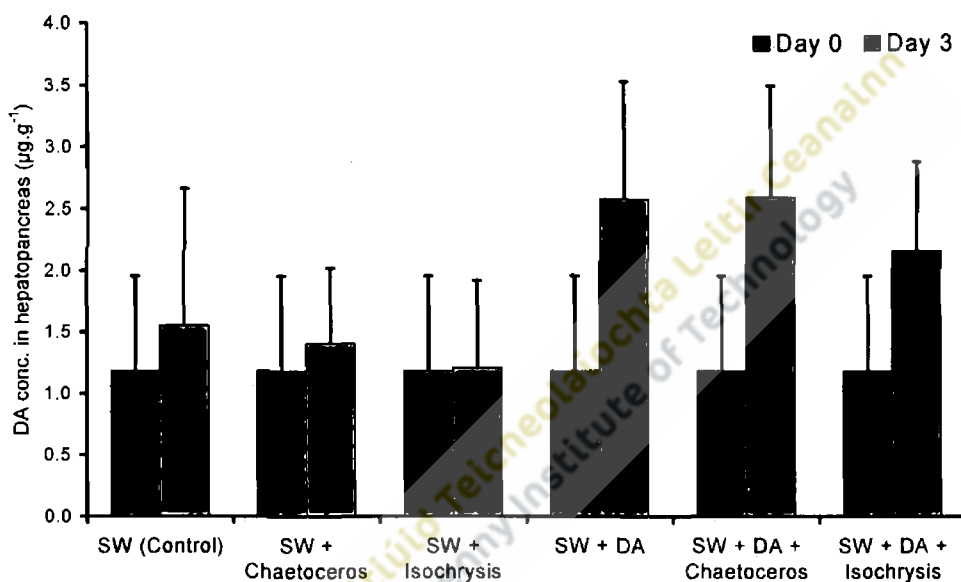


Figure 21. Design of the trial to investigate the uptake of dissolved DA.

Dissolved DA was not detected in the seawater used in the trial. Mean DA concentration of seawater in those tanks to which DA was added ranged from  $2.00 \pm 0.01 \mu\text{g.g}^{-1}$ . After 72 hours exposure all scallops were removed and each hepatopancreas analysed individually for DA concentration. Mean DA concentration in seawater of tanks containing toxin had decreased to  $1.93 \pm 0.02 \mu\text{g.g}^{-1}$ . Results from the trial on the uptake of dissolved DA from seawater are provided in Figure 22.



**Figure 22.** Uptake of dissolved DA in scallops after 72 hrs exposure to toxin.

The small increase in mean DA concentration in hepatopancreas from  $1.18 \pm 0.77 \mu\text{g.g}^{-1}$  to  $1.39 \pm 0.82 \mu\text{g.g}^{-1}$  at day three in scallops from the control tanks was attributed to experimental error. In tanks containing toxin, there was a significant increase in mean DA concentration in hepatopancreas to  $2.44 \pm 0.86 \mu\text{g.g}^{-1}$  after three days exposure ( $n = 72$ ,  $F = 25.95$ ,  $p = 0.000$ ). There were no significant differences in DA uptake in the presence of non-toxic diatoms ( $n = 24$ ,  $F = 0.002$ ,  $p = 0.967$ ) or flagellates ( $n = 24$ ,  $F = 1.152$ ,  $p = 0.231$ ) suggesting that non-toxic cells do not act as a vector for the transport of dissolved DA into scallops. Although scallops in the presence of toxin exhibited a significant uptake of dissolved DA from the water this must be balanced against exposure to a toxin concentration ranging from  $2.00$  to  $1.93 \mu\text{g.g}^{-1}$  over the 72-

hour duration of the trial. In this context, accumulation of dissolved DA from the water was considered negligible and perhaps only represents passive contamination of tissues exposed to the toxin rather than active uptake and sequestration of dissolved DA. However, the trial was conducted over a three-day duration, a time period possibly too short for significant DA uptake given that Douglas et al. (1997) in a feeding trial with toxic *Pseudo-nitzschia* cells reported that significant presence of DA in hepatopancreas only occurred after 6 days exposure.

In summary the studies in this research programme conducted with the objective of providing a better understanding of field variability demonstrated that there is little benefit in monitoring the cell density of total *Pseudo-nitzschia* species, though such data may support the development of an early warning system of possible impending toxicity. In the one site examined there was no evidence for the occurrence in the water column of thin layers of toxic *Pseudo-nitzschia* species or for benthic production of toxin. Uptake of dissolved DA from the water was negligible. These results are of importance for the design of new sampling programmes and modification of existing sampling programmes for the future.

## 6. Living with field variability

In this research programme the DA toxin concentration and the variability between king scallop tissues, between scallops of different sizes and between sampling sites located at different water depths in locations around the Irish coast have been quantified. Several factors that might contribute to an improved understanding of the causes of such variability have been examined. To assist in an assessment of the implications of these results for DA monitoring programmes, the most salient results of the study have been summarised below:

- **Variability in DA concentration between tissues**

Distribution of DA toxin between king scallop tissues was similar to that widely reported in the literature. Toxin concentrations have been considered below in terms of the levels in individual tissues and in the whole scallop since both are of significance in the context of EU regulations.

- ☐ **HEPATOPANCREAS**

This organ contains the majority of the DA toxin. The maximum DA concentration recorded was  $3834.4 \mu\text{g.g}^{-1}$  from Strangford Lough (Paper 5). Lowest concentrations were recorded off the east coast of the Isle of Man (Paper 4). Excluding those samples from the east coast of the Isle of Man with low DA concentration, high inter-animal variability in DA concentration was exhibited, values over the research programme ranging from 12.5 % to 82.5 %.

- ☐ **GONAD**

DA concentrations in composite samples of gonad tissue exceeded the regulatory limit of  $20 \mu\text{g.g}^{-1}$  at seventeen of the sixty-nine stations off the southeast coast of Ireland (Paper 2) and in seven of the twenty-two samples from the Isle of Man (Paper 4). The highest concentration of

61.3  $\mu\text{g.g}^{-1}$  was recorded in a sample from a station off the southeast coast (Paper 2). No DA concentration above the regulatory limit was recorded in Clew Bay (Paper 1, 3) or Kilkieran Bay (unpublished). Understanding changes in DA concentration in gonad during temporal studies was complicated by variations in gonad size during the reproductive cycle (Paper 1, 3).

□ ADDUCTOR MUSCLE

The DA concentration in composite adductor muscle samples was the lowest of any of the tissues analysed. Over the duration of the research programme the regulatory limit of 20  $\mu\text{g.g}^{-1}$  was only exceeded in three samples off the southeast coast of Ireland, the maximum concentration recorded being 31.8  $\mu\text{g.g}^{-1}$  (Paper 2). Concern was expressed regarding possible contamination of adductor during the freezing/thawing of frozen in-shell scallops or freezing/thawing of shucked scallops, whenever the hepatopancreas was retained.

□ WHOLE SCALLOP

Considerable variability in DA concentration of whole scallop was exhibited in the spatial distribution study off the southeast coast of Ireland (Paper 2). Toxin concentration in whole scallop exhibited good correlation with toxin concentration in hepatopancreas.

• **Variability in toxin concentration with scallop size**

From a scientific perspective scallop size can influence toxin concentration. One negative correlation and six positive correlations with scallop size were recorded in this research programme (Paper 1, 4, 5). The cause of these correlations was suggested as faster toxin accumulation and faster toxin depuration in smaller scallops respectively (Paper 1, 5). Perhaps the number of correlations of each type recorded in these studies reflects the faster rate of toxin accumulation and slower rate of toxin depuration reported in the literature. Considering only

scallops above a minimum commercial size of 100 mm, the size of scallop exhibited no correlation with DA concentration.

Variability within a sample, measured in terms of the CV, on any one date ranged from 21.9 % to 72.8 %, excluding the results from the east coast of the Isle of Man in which extraordinarily high CV's were attributed to the low DA concentrations recorded. Although mean toxin concentration in a sample was unrelated to scallop size, results suggested that there was higher variability measured in terms of CV's in smaller sized scallop (Paper 1) supporting the hypothesis that faster rates of accumulation and depuration occurred in smaller scallops. Higher CV's would increase the probability of obtaining an unrepresentative sample.

- **Variability in toxin concentration with water depth**

Different relationships between toxin concentration and water depth were recorded in different sites. DA concentration decreased with water depth off the southeast coast of Ireland and in Strangford Lough (Paper 2, 6), increased with water depth in Beirtrabui Bay (Bogan 2002) and showed no relationship with water depth around the Isle of Man (Paper 4). In those locations where the relationship between toxin concentration and water depth was examined on more than one occasion (Paper 2, 4, 6), the relationship reported remained consistent suggesting that it is a site-specific feature, possibly related to hydrodynamic characteristics at the site. No difference was recorded over a 12-month period between suspended and seabed scallops in terms of the DA concentration in hepatopancreas.

Mean DA concentration in hepatopancreas of samples at different depths exhibited CV's ranging from 39.4 % in Strangford Lough to 69.1 % in Beirtrabui Bay.



- **Understanding the causes of DA variability**

DA toxin concentration in hepatopancreas showed poor relationship with cell counts of *Pseudo-nitzschia* species. Cell counts exhibited sporadic deviations over periods of only one week.

Uptake of DA from solution was possible which might account for the poor relationship above, though further work is required to estimate the significance of the uptake of dissolved DA in influencing toxin concentration.

To assess the significance of the findings of this research programme in the context of toxin monitoring protocols used at a national and European level and recommendations that might ensue for DA toxin management, a series of questions and possible scenarios have been considered from the perspective of scientists, regulators, scallop farmers and fishermen.

The Food Safety Authority of Ireland (FSAI) has established two protocols for the management of ASP in Ireland depending on whether the scallops are captured from the wild fishery or produced by aquaculture. This differentiation dependent on the source of the scallops raises the question:

**Q. What constitutes a “scallop fishery” and what constitutes “scallop aquaculture”?**

Scallop farming practises used in Ireland comprise suspended culture in lantern nets from longlines, intensive seabed culture within a limited area or more extensive sowing culture often involving the re-stocking of a larger area or areas. Sometimes, diver-collected scallop are suspended from longlines for a period whilst a consignment is gathered, blurring the division between suspended and seabed culture. Differences in the scale of seabed farming practised raises the question of at what level extensive sowing culture should be regarded as a fishery and hence subjected to the same biotoxin protocols as

those applied to wild fishery management. By way of illustration, several examples are provided from the Irish industry of difficulties that might arise in interpretation of which protocol should be used. At Deegagh Point Shellfish Ltd. in Mulroy Bay and Inishoo Industries Ltd. in Clew Bay, scallops are currently farmed by suspended culture in lantern nets within a 2-hectare site and by seabed sowing within a 4-hectare site respectively. In both of these examples, the licensees have larger sites available for culture. At a larger scale, North West Shellfish Ltd. in Mulroy Bay have Aquaculture Licenses for sowing sites covering an area of approximately 300 hectares sub-divided into a total of ten different blocks, separated geographically by a distance of up to 9 km. One of the largest Aquaculture Licenses granted was that awarded to the South Connemara Shellfish Co-op. covering a total area of thousands of hectares and including both Kilkieran Bay and Beirtrabui Bay. Given the extent of spatial variability recorded in this research programme off the southeast coast of Ireland (Paper 2, 6) and the site specific variability with water depth off the southeast coast (Paper 2, 6), in Beirtrabui Bay (Bogan 2002) and in Strangford Lough (unpublished data), the question to be asked is whether the first two examples are really what the aquaculture protocol was designed to cover rather than the latter more extensive examples. In such extensive areas it is more likely that a sample for biotoxin monitoring will not be representative of the DA concentration in the entire area covered by the Aquaculture License and that as a consequence unacceptable risk might be more likely to arise. Adopting a precautionary approach to risk management of DA toxin yet retaining a sense of the practical nature of the aquaculture industry it is suggested that perhaps 50 hectares in a single block be considered as the maximum limit under which the “aquaculture” protocols are applied and that sites larger than this be subjected to wild fishery protocols. In the event that scientific data can be provided to confirm that such a limit is either too large or too small, then the proposed limit could be adjusted accordingly.

In this research programme, results have focussed on the DA concentration in the hepatopancreas, the tissue with the highest concentration of toxin, though levels in gonad, adductor muscle, tissue remainder and whole scallop have all been determined (Paper 1, 2, 3, 4, 5, 6). EU Decision 2002/226/EC refers to monitoring of the edible parts,

principally the adductor muscle and gonad. In some countries cell densities of *Pseudo-nitzschia* sp. have been used to provide an early warning system of the likelihood of toxicity levels being exceeded. Based on the data in this research programme the question is raised:

**Q. What should be monitored a) from a food safety perspective and b) for provision of an early warning system? Is there any value in monitoring hepatopancreas?**

From a food safety perspective; scientists, regulators, fishermen, farmers and processors unanimously agree that the edible parts, the adductor muscle and the gonad, which enter the human food chain, should be monitored. Given that most of the DA toxin accumulates in the hepatopancreas, an organ not consumed, the logic of basing regulations such as EU Decision 2002/226 on whole scallop concentrations whereby highly toxic non-edible parts were homogenised with low toxin edible parts, would seem inappropriate.

From a monitoring perspective for the provision of an early warning system, advantages and disadvantages were identified with each tissue. Adductor muscle, though a tissue entering the food chain and therefore of prime importance, often exhibited low concentrations of toxin making measurement more difficult. The risk of contamination due to seepage of tissue fluid from the highly toxic hepatopancreas was also considered more likely. Low toxin concentrations resulted in high CV's and hence reduced confidence in the determination of trends in toxin concentration based on this tissue. Gonad tissue, though exhibiting higher toxin concentration and thus greater confidence in results based on this tissue than adductor muscle, was disadvantaged by difficulties in interpreting results due to changes in size associated with the reproductive cycle (Paper 1, 2, 3, 4). Monitoring of toxin concentrations in hepatopancreas were somewhat easier than in the other tissues due to the high concentrations present and this tissue showed only minor seasonal variation in size compared to gonad (Paper 1, 3). However, toxin monitoring based on hepatopancreas tissue was disadvantaged since this tissue is not

consumed and showed no relationship with gonad toxin concentrations due to variability in the size of the latter organ. The hepatopancreas of scallops shucked from the shell should be removed prior to freezing or dispatch to the laboratory to reduce the risk of contamination due to toxin seepage (Paper 2, 6). Using the toxin concentration in whole scallop for monitoring of toxin trends would seem inappropriate for similar reasons to those given previously and in addition homogenisation of whole tissue is more difficult practically, requiring a powerful homogeniser to address the stringy nature of the mantle edge.

Some EU Member States have adopted Decision 2002/226/EC and use an area-based approach to DA toxin management whereby determination of whether a fishing area remains open or closed for harvesting depends on the DA concentration from a single sample of 10 scallops. Under this approach, if the DA concentration of the sample is below  $20 \mu\text{g.g}^{-1}$  then the area is open for harvesting, if above  $250 \mu\text{g.g}^{-1}$  then the area is closed for harvesting and if between  $20 - 250 \mu\text{g.g}^{-1}$  the edible parts of the sample must be tested and the DA concentration must be below  $4.6 \mu\text{g.g}^{-1}$  to allow harvesting to proceed. All scallops harvested in this latter situation must be processed and the edible parts to be placed on the market must have a DA concentration below  $20 \mu\text{g.g}^{-1}$ . In Ireland, a landings-based approach to DA toxin management has been adopted whereby samples of all landings from the wild fishery are analysed. The approach adopted in Ireland has necessitated that all scallops from the wild fishery be processed. If edible tissues are below  $20 \mu\text{g.g}^{-1}$  then processed scallops are allowed onto the market and if above  $20 \mu\text{g.g}^{-1}$  then processed tissue is destroyed. These two different approaches raise the question:

**Q. Should Ireland and other countries use a DA biotoxin management approach based on scallop landings or one based on fishery areas?**

Both approaches have advantages and disadvantages that should be considered in selecting the most appropriate tool for DA toxin management.

An area-based approach demands that boats be available for procuring samples for analysis on a routine basis from each of the different fishery areas. Such an approach assumes that scallops exhibit no spatial variation within a sampling area, an invalid assumption based on the results reported in this research programme (Paper 2, 4, 6). The size of the area considered as a homogeneous unit was not addressed in EU Decision 2002/226. The relationship between toxin concentration and water depth, shown to vary between sites in this research programme (Bogan 2002, Paper 2, 4, 6), is ignored in implementing the area-based approach used in EU Decision 2002/226. Major concerns based on whether the sample taken from an area is representative of the whole area arise, particularly when toxin concentrations approach the  $20 \mu\text{g.g}^{-1}$  level. The time between sampling of an area and the reporting of results may become an issue if consignments entering the marketplace before results are announced are to be avoided. Advantages of the area-based approach if implemented as intended in EU Decision 2002/226 are that some indication is available to fishermen, prior to departing for the scallop fishing grounds, of the future market for their catch. Importantly, access to the more valuable fresh scallop market has not been curtailed by the area-based landings approach.

An approach to DA toxin management based on sampling of each scallop landing overcomes the problem of spatial variability. Issues associated with the extent of the area regarded as a homogeneous unit no longer arise (Paper 2, 4, 6), and effects associated with water depth (Paper 2, 3, 4, 6) are eliminated from the risk analysis. Agencies charged with responsibility for monitoring DA concentration need neither sampling vessels nor crew. Since each scallop landing is sampled and analysed, a reduced risk of contaminated product entering the food chain should occur. Disadvantages of such an approach are the increased throughput required of toxin testing laboratories and the absence of advice for fishermen prior to their departure for the fishing beds. One key concern that has arisen from the manner in which the landings-based approach has been implemented in Ireland has been the requirement that all scallops from the wild fishery be processed thus preventing access to the higher-value, fresh scallop market. For example, if DA concentrations of  $17 \mu\text{g.g}^{-1}$  in the gonad and  $1.7 \mu\text{g.g}^{-1}$  in the adductor muscle are reported, why should such scallops be consigned to processing and access to the more

valuable fresh market denied? Results from this research programme (Paper 2, 3, 4, 6) support the case for a landings-based approach to DA toxin management to reduce the risk of contaminated product entering the food chain. To address the question of access to the higher-value, fresh market, particularly in areas where significant landings from the scallop fishery occur, raised the question:

**Q. Can a case be made for scallop fishermen in the southeast, landing scallops from the southeast fishery or from the east coast of Isle of Man to support access to the fresh scallop market?**

The majority of scallop landings in Ireland occur in the southeast ports of Kilmore Quay, Dunmore East and Waterford. Harvesting used to be focussed on the southeast scallop beds but now extends throughout the southern Irish Sea, Isle of Man, western English Channel and northwest of Brittany (Tully et al. 2002). Two investigations off the southeast coast reported low DA toxicity in scallops from offshore grounds (Paper 2, 6). Three studies of DA toxin concentrations in scallops from beds around the Isle of Man suggested that scallops from the east coast beds – Laxey and East Douglas, had exceptionally low DA toxin concentrations (Paper 4). Maintaining a landings-based approach to DA toxin management, access to the fresh market would only be possible if fast turnaround of samples for DA analysis could be achieved such that suitable packing, transport and dispatch to the fresh market could be arranged. Several options were considered which might facilitate a fast sample throughput:

- Field Use of ASP ELISA (BioSense®)

This commercially available kit for DA analysis based on the competitive direct Enzyme Linked Immunosorbent Assay (ELISA) principle has been reported to be suitable for the analysis of a variety of shellfish matrices, body fluids of marine mammals and for algal and sea water samples (Garthwaite et al. 2001, Samdal et al. 2005). The test kit available in a 96-well format and suitable for 36 samples has recently been reformatted into an 8 x 12 strip-well format, so that an entire plate does not need to be analysed on each sampling

occasion. In this research programme samples of scallop hepatopancreas, gonad, adductor muscle and remainder from 64 sampling stations off the southeast coast of Ireland were analysed by ASP ELISA kit and results compared with the approved HPLC method (Bender 2004, Bender et al. 2004). The assay procedure for this direct cELISA procedure is summarised below.

Preparation of buffers, reagents, standards and sample dilutions

↓  
1<sup>st</sup> washing step

↓  
Addition of sample/standard dilutions to wells

↓  
Addition of anti-HRP conjugate

↓  
1<sup>st</sup> incubation step

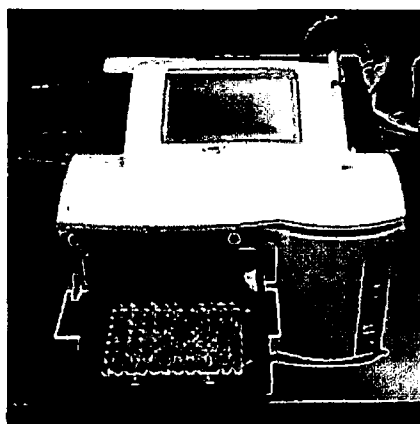
↓  
2<sup>nd</sup> washing step

↓  
Addition of TMB

↓  
2<sup>nd</sup> incubation step (development of plate)

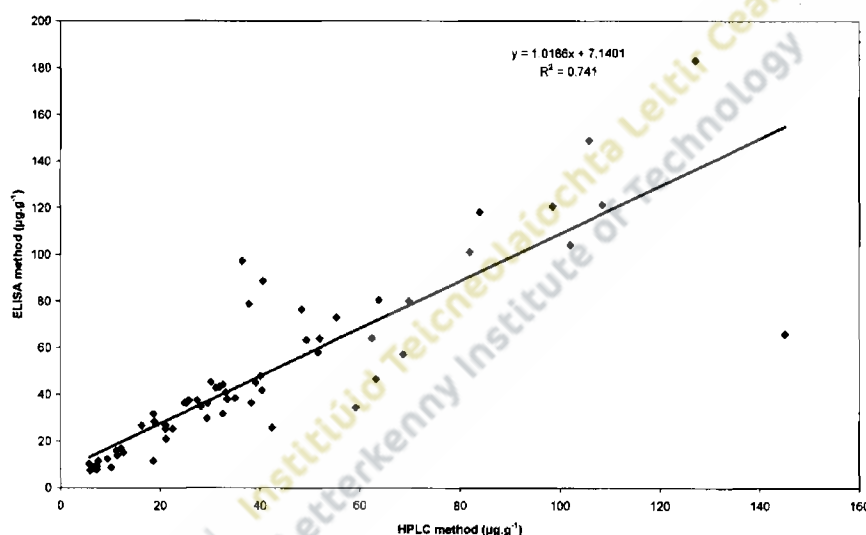
↓  
Addition of H<sub>2</sub>SO<sub>4</sub>

↓  
Measure absorbance at 450nm in micro-plate reader





DA concentrations in whole scallop determined by the ELISA and HPLC methods exhibited a highly significant correlation ( $R^2 = 0.74$ ,  $n = 64$ ) (Figure 23), with the slope of the linear correlation exhibiting a bias of approximately 2 % (Bender et al. 2004). According to Samdal et al. (2005) the specificity of the ELISA method for the detection of yessotoxins in blue mussels, *Mytilus edulis*, could be a disadvantage, since the ELISA failed to distinguish between toxic and non-toxic analogues, leading to discrepancies between the two methods. Similarly with DA, the ELISA kit detects both DA and all of the isomers simultaneously whilst the HPLC method distinguishes between DA, epi DA and other isomers and this may have contributed to the bias reported.



**Figure 23.** Correlation between HPLC and ELISA results for DA determination in whole scallop samples from the southeast fishery ( $\mu\text{g.g}^{-1}$ ).

DA concentrations in whole scallop determined by ELISA were below  $20 \mu\text{g.g}^{-1}$  at 15 sampling stations (23.4 %) and above  $20 \mu\text{g.g}^{-1}$  at 49 stations (76.6 %). By comparison, DA concentrations in whole scallop determined by HPLC were below  $20 \mu\text{g.g}^{-1}$  at 18 sampling stations (28.1 %) and above  $20 \mu\text{g.g}^{-1}$  at 46 stations (71.9 %).

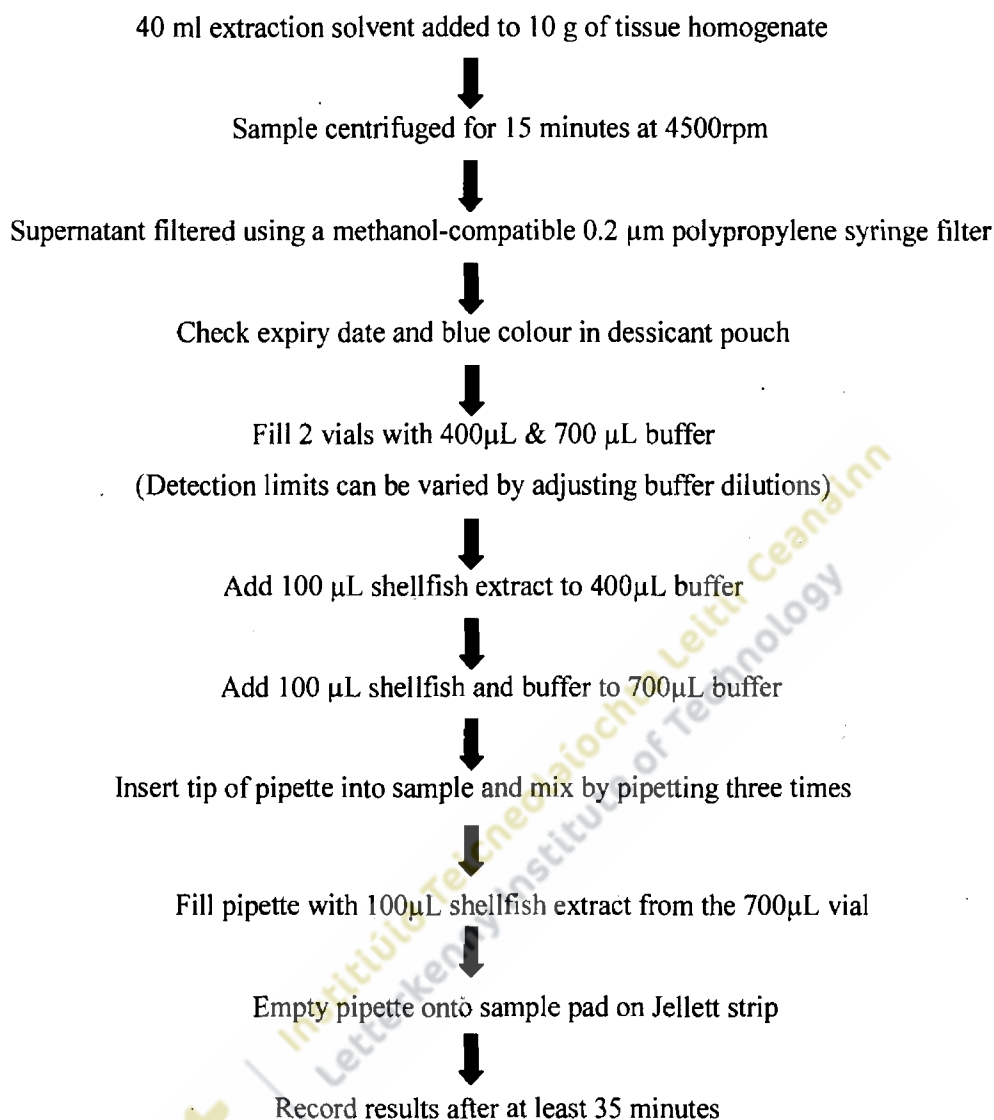
Correlations between DA concentrations in individual scallop tissues determined using the two techniques were also examined. The correlation between DA concentration in the adductor muscle determined by ELISA and HPLC exhibited the highest correlation

( $R^2 = 0.91$ ). Correlations between the two techniques were lower with hepatopancreas tissue ( $R^2 = 0.77$ ) and remainder tissue ( $R^2 = 0.64$ ). A low correlation was exhibited between the two techniques with gonad tissue ( $R^2 = 0.39$ ) indicating a sample matrix effect on the performance of the ELISA technique (Bender et al. 2004). Overall the ELISA technique was easy to use and deemed acceptable as a screening tool. The major drawbacks of the ELISA technique were identified as the time-consuming sample dilutions that extended the preparation time considerably and the need for a micro-plate reader. The high sensitivity of the ELISA technique was deemed unnecessary for routine monitoring work with scallop tissues.

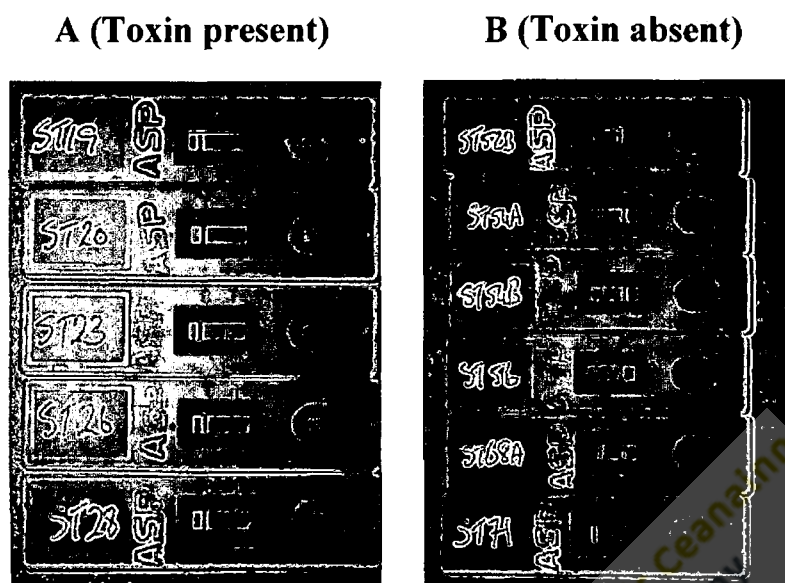
- Field use of Jellett Rapid® Test kit for ASP

The Jellett Rapid® Test kit for ASP is another immunological test kit available commercially in a lateral flow format. Similar kits have been developed for PSP and more recently DSP, though the latter is not yet available for commercial use. The Jellett Rapid® test kit for PSP has been shown to be highly effective for the detection of PSP toxins in shellfish (Laycock et al. 2000) and in plankton matrices (Silva et al. 2001). The kits have been reported to be suitable for semi-quantitation as a screening tool, providing a positive or negative (Yes/No) result (Jellett et al. 2002).

In this research programme the DA concentration of 53 samples of whole scallop, *Pecten maximus* was determined by HPLC and Jellett Rapid® kits (Paper 8). The assay procedure for the Jellett kit is summarised below for detection at the  $10 \mu\text{g.g}^{-1}$  limit.

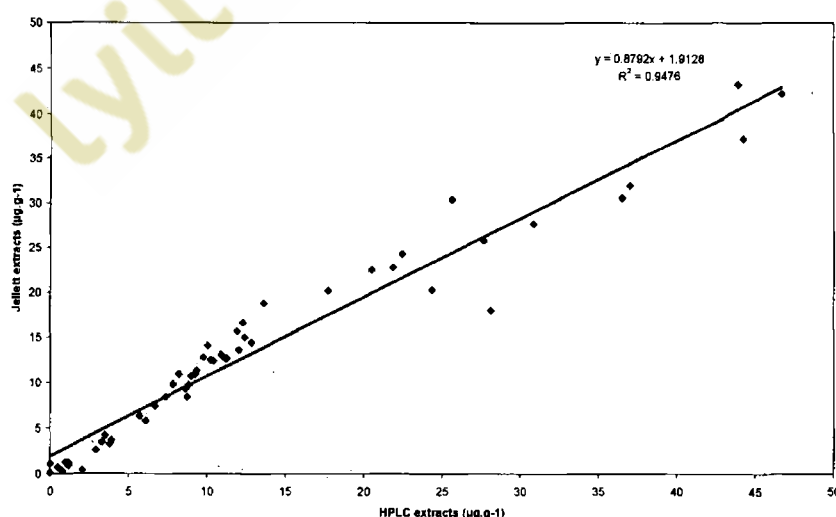


The Jellett Rapid<sup>®</sup> test kit for ASP provides a visual determination of the presence or absence of DA in the shellfish sample. To ensure that the test strip is functioning correctly a C line (Control line) must always appear on the strip. Absence of a T (Toxin) line (T = 0 % of C line) or a faint shadow at the position of the T line indicates the presence of DA toxin. When no DA toxin is present in the sample, the T line may be more intense than the C line (T >100 % of C line), as intense as the C line (T =100 % of the C line), or as low as 25 % of the C line. Examples of positive results (toxin present) and negative results (toxin absent) with the Jellett Rapid<sup>®</sup> test Kit are provided in Figure 24.



**Figure 24.** Results from the Jellett Rapid® test kit for ASP. The C (Control) line is located on the left of the observation window and the T (Toxin) line on the right of the observation window. The samples in column A exhibiting no T line or a faint T line are positive for DA. The samples in column B exhibiting a visible T line are negative for DA.

DA extraction procedures using the validated HPLC extraction procedure and the Jellett extraction procedure were compared by HPLC. DA concentrations from both extraction procedures exhibited a good linear relationship ( $R^2 = 0.95$ ), indicating that both extraction procedures were in good general agreement (Figure 25).



**Figure 25.** DA concentration of HPLC extracts and Jellett kit extracts analysed by HPLC.

The Jellett Rapid<sup>®</sup> test kit for ASP detected DA in all samples containing the toxin above the EU regulatory limit of 20  $\mu\text{g.g}^{-1}$ . Good reproducibility was observed between the test kits when used in triplicate (Paper 8). As the Jellett Rapid<sup>®</sup> kit is marketed as having the potential to be operated in the field, results were also determined in the processing factory and compared to results from the laboratory. DA toxin results of whole scallop tissues from 53 sampling stations off the southeast coast determined by the Jellett Rapid<sup>®</sup> kit (Yes/No) in the laboratory showed 76 % agreement with results determined in the field (processing factory) (Paper 8). The detection limit of the Jellett Rapid<sup>®</sup> kit for ASP is reported as being between 8  $\mu\text{g.g}^{-1}$  to 12  $\mu\text{g.g}^{-1}$  (Jellett et al. 2002), although DA concentrations below this level were detected in some samples. Interpretation of results from the Jellett kit based on the intensity of the T line compared to the C line is a somewhat subjective process and is considered to be the principle cause of differences in the results between analysts (Paper 8).

- Establish a port-based HPLC testing facility in the southeast

Although both immunological techniques exhibited potential for the future, HPLC is currently the only technique approved for use in the EU for the determination of DA in shellfish. Since it is the responsibility of the Competent Authority in each Member State to ensure that shellfish are safe to be placed on the market, the only feasible way to allow access to the fresh market is if a State-operated, HPLC testing facility existed in the port to allow rapid turnover of samples and potentially access to the fresh market. A private testing facility operated by a Fishermen's Co-operative would be inappropriate on the basis that responsibility for product testing rested outside the Competent Authority, that self-regulation by an industry would be undesirable and that vested interests were participating in the toxin testing process. A testing facility established by a Fishermen's Co-operative, but operated by the State, would allow rapid testing of scallops from the wild fishery and might be the way forward to support access to the fresh scallop market.

Compared to the validated HPLC technique, both immunoassay procedures were simple to perform although the ELISA procedure was deemed to be more time consuming

(Bender 2004). Neither the ELISA kit or the Jellett kit required expensive equipment and the consumable materials required for use with either method could easily be stored on board a fishing boat or in a processing factory. Comparison of both procedures in the context of operating time and cost per sample has been provided in Table 6.

Method of Analysis	HPLC	ELISA	Jellett
Sample preparation time (mins)	30	180	20
Calibration time (mins)	180	n/a	n/a
Measurement time (mins)	30	1	35
Detection limit ( $\mu\text{g.g}^{-1}$ )	1.0	0.03	8 - 12
Cost per sample (€)	150	275	18

**Table 6.** Comparison and evaluation of the HPLC regulatory method with the BioSense® ELISA kit and Jellett kits.

Both immunological kits demonstrated potential for use as field methods. The analytical cost per sample varied substantially between the different methods. The most expensive technique, the BioSense® ELISA kit, is designed for the analysis of 36 samples and costs €275 per plate. This kit has recently been re-formatted into 8 x 12 well strips so that costs per sample can be reduced. The extremely low detection limit of the BioSense® ELISA method was deemed unnecessary for routine monitoring of DA in shellfish tissues, however the low detection limits suggest that the kit may have potential use for the determination of DA in algal cells or seawater samples as part of an early warning monitoring system.

The least expensive technique, the Jellett Rapid® kit for ASP is a semi-quantitative test and has potential as a screening tool to reduce the number of samples that must be analysed by the regulatory HPLC method. Mackintosh and Smith (2002) reported that this ASP kit was too sensitive to be used as a screening method and that a detection limit

closer to 20  $\mu\text{g.g}^{-1}$  would be advantageous. Despite the low cost per sample and the ease of use, the main drawback of this kit originated from the subjective assessment of the intensity of the T line relative to the C line and hence errors in interpreting a positive or negative result, particularly near the limit of detection. Recently an even more sensitive version of the Jellett kit has been developed which, as with the BioSense® ELISA kit, may have a role in the determination of DA in algal cells or seawater samples as part of an early warning system.

Given the need for an advisory early warning system of impending DA toxicity raised the question:

**Q. What techniques might best constitute such a system?**

The absence of a correlation between DA toxicity and the abundance of *Pseudo-nitzschia* species over a 24-month duration in Clew Bay suggested that there is limited practical value in phytoplankton monitoring based on total *Pseudo-nitzschia* cell counts. Although DNA-based techniques have been developed for toxic *Pseudo-nitzschia* species present in other parts of the world, such techniques have not been employed routinely to date in Irish waters. The considerable fluctuations in total *Pseudo-nitzschia* cell counts from one sample to the next reported in the Clew Bay study suggests that further emphasis should be given to developing an improved sampling technique or sampling protocol if a proactive early warning system based on phytoplankton is to be developed.

Both the highly sensitive BioSense® ELISA kit and the recently introduced Jellett Rapid® kit for ASP with lower detection limit may have potential for the future in contributing to the provision of an early warning system for DA. Investigations need to be undertaken of the correlation between DA toxicity in scallop and DA toxin levels in seawater to determine the potential of these two immunological techniques in contributing to an early warning system.



Given the preliminary data that suggested that scallops are capable to some extent of the uptake of DA dissolved in water (Paper 7), a SPATT system using an inert resin for absorption of this hydrophilic toxin might have more potential than either of the two previous techniques in the provision of an early warning system (MacKenzie et al. 2004).

Once intoxicated with DA, depuration from king scallops was demonstrated to be a slow, prolonged process (Paper 1). Interest in accelerating the rate of toxin depuration in scallop has raised the question of whether:

**Q. Holding scallops in lantern nets suspended off the seabed might enhance the rate of depuration?**

Comparative DA concentrations in scallops held in lantern nets and on the seabed in Clew Bay over a period of 12 months exhibited no discernible difference in toxin concentration of the hepatopancreas, the main organ in which DA is accumulated (Paper 3). Although not a trial designed to investigate the rate of depuration, the data suggested that there would be no merit in proceeding with such an approach to enhancing depuration rates.

Assuming that scallops of commercial size are used for DA testing, there was only limited evidence to suggest that the shell length of scallops had an influence on mean DA concentration in the hepatopancreas (Paper 1, 2, 4, 5). This raised the question:

**Q. Is the size of commercial scallops provided for analysis irrelevant?**

Based on the CV's of DA concentration in hepatopancreas, the results suggested that less variability occurred in samples comprising larger scallops than smaller scallops (Paper 1, 2, 5). Such a result might be anticipated given the data indicating faster rates of DA uptake and depuration in smaller scallops. Reduced variability in DA concentration between scallops in a sample would be advantageous from both a risk-management perspective – reduced risk of contaminated product being allowed into the food chain and

from a fishing perspective – reduced incidence of false closures if an area-based approach to biotoxin monitoring were being used. Even if a landings-based approach to biotoxin management is used, the data suggest that there is some merit to using the largest scallops available for DA toxin monitoring.

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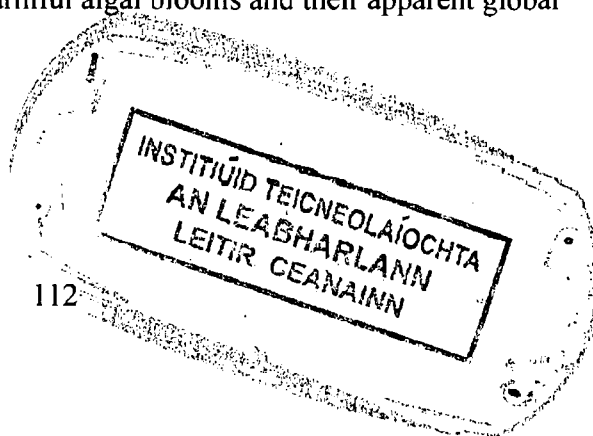
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Appendices



## Appendix 1. Paper 1

The influence of size on domoic acid concentration in king scallop,  
*Pecten maximus* (L.). Harmful Algae.

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J.W.S. and P.H. designed the research programme.

Y.M.B. and A.L.H. prepared the scallop tissues.

Y.M.B. performed the research study.

J.G. provided technical support.

Y.M.B., J.W.S. and D.J.K. analyzed the data.

Y.M.B., J.W.S. and P.H. prepared the manuscript.

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## The influence of size on domoic acid concentration in king scallop, *Pecten maximus* (L.)

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### Abstract

Concentrations of domoic acid (DA), the biotoxin responsible for amnesic shellfish poisoning (ASP), exceeding the regulatory limit of  $20 \mu\text{g g}^{-1}$  have caused restricted harvesting and closures of wild king scallop fisheries. Toxin monitoring programmes have reported significant inter-animal variation in DA concentration between scallops from the same area. For the development of reliable sampling and management protocols an understanding of the magnitude and causes of inter-animal variation in toxin concentration are important. Ten samples were collected from an aquaculture site in Clew Bay, Co. Mayo off the west coast of Ireland between February 2003 and February 2004, each sample comprising 12 scallops of each of the following size groups: small (70–85 mm), medium (85–100 mm), large (100–115 mm) and very large ( $>115$  mm). DA concentration in each hepatopancreas and in composite samples of both gonad and adductor muscle from each size group on each sampling occasion were measured. High inter-animal variability in DA concentration in hepatopancreas was recorded; CVs ranging from 12.5% to 82.5%. One negative correlation ( $R^2 = 0.7079$ ) between DA concentration in hepatopancreas and scallop shell length, three positive but weak correlations ( $R^2 = 0.4536, 0.3459$  and  $0.4665$ ) and six no correlations were exhibited. Negative correlations were attributed to faster DA uptake by smaller scallops, positive correlations to faster DA depuration by smaller scallops. If only scallops greater than or equal to 100 mm shell length, the minimum commercial size of this species were considered, no correlation occurred on any of the 10 sampling occasions.

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**Keywords:** Domoic acid; Inter-animal variability; *Pecten maximus*; Scallop

### 1. Introduction

Production of the biotoxin, domoic acid (DA), has been reported in 10 species of diatoms within the genus *Pseudo-nitzschia* (Bates, 2000). Filter feeding bivalves such as scallops and mussels consuming these toxin-producing phytoplankton species can accumulate DA to high concentrations (Zaman et al., 1997). Human

consumption of shellfish contaminated with DA results in amnesic shellfish poisoning (ASP). This condition was first recorded in Canada in 1987 when over 100 people became ill after consuming mussels *Mytilus edulis* contaminated with the toxin. Early symptoms included nausea, gastroenteritis and vomiting followed within 48 h by neurological symptoms such as confusion, lethargy, disorientation and memory loss that could persist indefinitely; three of those affected in this outbreak died (Quilliam and Wright, 1989; Todd, 1993).

Since this initial toxic event in blue mussel, *M. edulis* numerous molluscs and crustaceans have been

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shown to accumulate this algal toxin (Arévalo et al., 1998; Vale and Sampayo, 2001, 2002; Amzil et al., 2001; Wekell et al., 2002; Powell et al., 2002; Bargu et al., 2003; Costa et al., 2003, 2005; Kaniou-Grigoriadou et al., 2005; James et al., 2005). In European waters, DA toxicity has impacted particularly on king scallop fisheries where accumulation and prolonged retention of toxin in this species has resulted in extensive harvesting closures, perhaps the most significant being those applied in Scotland (Arévalo et al., 1998; Gallacher et al., 2001; Smith et al., 2006). To ensure the safety of shellfish for human consumption EU Amendment 97/61 to Directive 91/492/EEC established the maximum allowable concentration of DA in whole shellfish or edible parts as  $20 \mu\text{g g}^{-1}$ . Localisation of the majority of toxin in the scallop hepatopancreas, slow depuration rates leading to extended periods of fishery closure and high inter-animal variation between scallops led to EU Commission Decision 2002/226/EC which allowed harvesting of scallops with a whole body DA concentration exceeding  $20 \mu\text{g g}^{-1}$  if the parts to be marketed, principally the adductor muscle and gonad, contained less than  $4.6 \mu\text{g g}^{-1}$ . In those countries where Commission Decision 2002/226/EC has been implemented, decisions on area management of harvesting areas, viz. whether an area remains open or closed for harvesting is based on DA concentration from a single sample of 10 scallops. Concern regarding the high levels of inter-animal variability in DA concentration between scallops from the same area and lack of understanding of the causes of such variation has been well documented (Arévalo et al., 1998; Campbell et al., 2001; Gallacher et al., 2001; Fryer et al., 2002; Bogan et al., 2006a).

In Ireland, closure of scallop harvesting areas due to ASP toxin first occurred in December 1999 and extended in some areas for several months. The current management scheme applied to this biotoxin in scallops from the wild fishery necessitates that all the catch be shucked to remove the hepatopancreas and edible parts frozen prior to testing. Neither adductor muscle nor gonad is allowed to enter the marketplace before test results indicate that DA concentration in these parts is below  $20 \mu\text{g g}^{-1}$ . This landings-based approach has allowed resumption of harvesting from fishing grounds that otherwise would have remained closed under amendment 97/61 of EU Directive 91/492/EEC, but has necessitated that all the catch be processed prior to sale. Concerns regarding inter-animal variability in DA concentration between scallops from the same area and lack of understanding of the causes of such

variation remains as an issue despite the differences in toxin management schemes.

The contribution to inter-animal variability in DA concentration of many factors including the magnitude, persistence and exposure to toxic phytoplankton, geographical location, water depth and currents, nature of the seabed, shell size/body mass and differences in feeding rates has been considered (Aalvik and Framstad, 1981; White et al., 1993; Arévalo et al., 1998; Bricelj et al., 1990; Bricelj and Shumway, 1998; Powell et al., 2002; Strohmeier et al., 2005; Costa et al., 2005). Amendment 97/61 of EU Directive 91/492/EEC and Commission Decision 2002/226 do not refer directly to the size of shellfish to be used for DA monitoring, but makes reference to bivalve molluscs at the 'harvesting stage'. For the development of reliable sampling and management protocols, irrespective of whether area-based or landings-based schemes are applied, it is essential to know if the size of scallops has any influence on toxin concentration. The objectives of this study were to quantify inter-animal variation in DA concentration in king scallop and to determine if there was any relationship with scallop size.

## 2. Materials and methods

Samples of king scallop *Pecten maximus* were collected by diving from a seabed aquaculture site in Clew Bay, County Mayo off the west coast of Ireland at approximately monthly intervals from February 2003 to February 2004 (Fig. 1). All samples were collected from a depth of approximately 12–15 m and comprised 12 individuals in each of the following size groups: small (70–85 mm), medium (85–100 mm), large (100–115 mm) and very large (>115 mm) based on scallop shell length except for the first sample (26 February 2003) from which the very large size group was not available. On several occasions one or two shells in a size group were empty upon arrival at the laboratory.

Following measurement of shell dimensions and determination of age using ring analysis, scallops were dissected and the weight of total soft tissue and individual weight of the hepatopancreas, gonad and adductor muscle recorded. Individual analysis of the DA concentration in each hepatopancreas was performed. Composite samples of gonad and composite samples of adductor muscle from each size group on each date were prepared for analysis. Sub-samples of composite tissues were analysed in triplicate for DA toxin concentration.

DA was extracted from scallop tissue samples using procedures based on Quilliam et al. (1995) with several

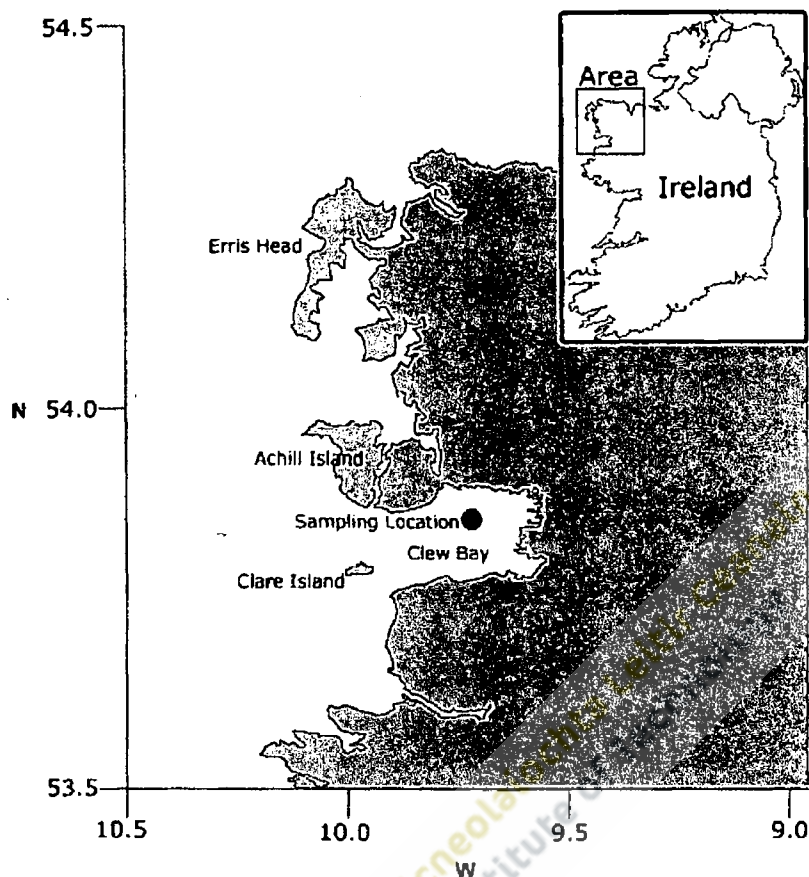


Fig. 1. Sampling location in Clew Bay, County Mayo, Ireland.

modifications. Extraction was performed from approximately 4.0 g of tissue homogenate with 16 ml of 50:50 extraction solvent (methanol:water) in a blender (Ultra Turrax T25, IKA®-Works) for 4 min at high speed. DA was extracted from each hepatopancreas individually. Where hepatopancreas weight exceeded 4.0 g, the organ was homogenised and a 4.0 g sub-sample used for extraction. Following extraction, homogenates were centrifuged at 3800 rpm for 30 min. A sample of supernatant was filtered using a methanol-compatible 0.45  $\mu\text{m}$  syringe filter and the combined concentration of DA and *epi*-DA in the filtered extract determined using a Shimadzu HPLC/UV and following equipment upgrade a Shimadzu HPLC/DAD (Mason Technology, Dublin). In those instances where the combined DA concentration exceeded that of the highest standard, the filtered extract was diluted with extraction solvent and the measurement of DA and *epi*-DA repeated. All solvents used were HPLC grade (Lennox Lab. Suppliers, Dublin). Mobile phase consisted of 10% acetonitrile and 1% TFA (trifluoroacetic acid) prepared with deionised water. HPLC flow rate was 0.5 ml min<sup>-1</sup>

and injection volume 20  $\mu\text{l}$ . Calibration was performed externally using six DA calibration standards between 0.2 and 10  $\mu\text{g ml}^{-1}$  prepared from Certified Reference Standard obtained from the NRC, Canada. Calibration curves were prepared for each sample batch and were always linear ( $R^2 > 0.999$ ).

Statistical analysis of all data was performed using SPSS, Version 12.

### 3. Results

#### 3.1. Toxin distribution in scallop tissues

Over the study duration the hepatopancreas of the king scallop proved to be the major location for ASP toxin, followed by gonad with generally 1–2% of the toxin burden, and only trace levels detected in adductor muscle. The number of scallop analysed in each size group on each sampling date together with the mean, standard deviation and relative standard deviation (R.S.D.) of DA concentration in hepatopancreas are provided in Table 1. Mean DA concentration in



Table 1  
Concentration of domoic acid in hepatopancreas of four size groups of king scallop from February 2003 to February 2004

	Small			Medium			Large			Very large		
	n	Mean ± S.D. (µg g <sup>-1</sup> )	R.S.D. (%)	n	Mean ± S.D. (µg g <sup>-1</sup> )	R.S.D. (%)	n	Mean ± S.D. (µg g <sup>-1</sup> )	R.S.D. (%)	n	Mean ± S.D. (µg g <sup>-1</sup> )	R.S.D. (%)
26 February 2003	10	1066.8 ± 242.5	22.7	12	323.2 ± 68.6	21.2	12	272.6 ± 59.8	21.9	12	599.5 ± 234.1	39.0
3 April 2003	11	330.8 ± 272.8	82.5	12	452.3 ± 200.4	44.3	11	698.0 ± 156.6	22.4	12	524.0 ± 65.5	12.5
23 May 2003	12	414.0 ± 112.7	27.2	12	467.4 ± 167.2	35.8	12	457.7 ± 134.6	29.4	12	261.2 ± 89.4	34.2
3 July 2003	11	228.6 ± 89.5	39.1	12	209.5 ± 72.6	34.7	12	216.5 ± 107.2	49.5	11	364.9 ± 75.5	20.7
30 July 2003	12	277.3 ± 83.3	30.0	11	297.3 ± 44.7	15.0	12	332.7 ± 140.6	42.3	12	245.0 ± 59.5	24.3
3 September 2003	12	220.8 ± 70.1	31.7	12	219.7 ± 84.8	38.6	12	305.0 ± 69.4	22.8	12	210.5 ± 47.6	22.6
25 September 2003	12	236.3 ± 83.8	35.5	12	226.2 ± 81.9	36.2	12	223.7 ± 65.4	29.2	12	270.4 ± 109.1	40.3
12 November 2003	12	81.1 ± 38.8	47.8	12	186.3 ± 86.5	46.4	12	298.3 ± 89.4	30.0	12	241.5 ± 69.8	28.9
4 December 2003	12	59.9 ± 24.6	41.1	12	230.3 ± 90.7	39.4	12	239.0 ± 109.4	45.8	12	211.8 ± 62.0	29.3
11 February 2004	12	49.6 ± 15.9	32.0	11	170.9 ± 42.5	24.9	11	245.2 ± 43.8	17.8			

hepatopancreas exceeded the regulatory concentration of 20 µg g<sup>-1</sup> in all size groups from all samples and ranged from a maximum of 1066.8 ± 242.5 µg g<sup>-1</sup> to a minimum of 49.6 ± 15.9 µg g<sup>-1</sup>. The highest concentration of DA recorded in hepatopancreas of an individual scallop was 1348.1 µg g<sup>-1</sup> in this study. Considerable inter-animal variation of DA concentration in hepatopancreas in sample batches was detected, R.S.D.s ranged from a minimum of 12.5% to a maximum of 82.5%.

The mean, standard deviation and R.S.D. of DA concentration in gonad samples of each size group on each sampling date, based on triplicate analysis of a composite of gonad tissue, are shown in Table 2. DA concentration in gonad ranged from a maximum of 9.18 ± 0.15 µg g<sup>-1</sup> to a minimum of 0.16 ± 0.00 µg g<sup>-1</sup> over the study duration. On several occasions the gonad composite from small-sized scallops provided sufficient tissue for only one 4 g sample for analysis. The highest concentration of DA recorded in a gonad composite sample was 9.59 µg g<sup>-1</sup>. Higher than anticipated R.S.D.s recorded in some instances were attributed to the low concentrations of DA present and poor tissue homogeneity due to inadequate blending of the limited tissue available.

DA concentrations in composite samples of adductor muscle never exceeded the limit of quantification (LOQ = 1.00 µg g<sup>-1</sup>).

### 3.2. Variation with scallop size

During the study from February 2003 to February 2004, mean shell length and standard deviation of scallops in the four size groups were as follows: small 81.63 ± 6.91 mm, medium 96.92 ± 5.61 mm, large 108.75 ± 3.90 mm and very large 119.98 ± 5.07 mm. Scallop shell length, shell height, shell depth, total tissue weight and weight of hepatopancreas, gonad and adductor muscle were recorded for each scallop. Individual analysis of the hepatopancreas of each scallop was performed to provide data on variability in DA concentration within each sample batch, to allow comparison of variability both within a sample, within a size group and between size groups to highlight any significant differences between small, medium, large and very large scallops. Triplicate analysis of composite gonad and composite adductor muscle samples of each sample batch were performed because preliminary studies suggested that differences in DA concentration between scallops of different size, if present, would be more difficult to confirm in these tissues due to the lower concentrations present.

Table 2  
Concentration of domoic acid in gonad of four size groups of king scallop from February 2003 to February 2004

	Small		Medium		Large		Very large	
	Mean ± S.D. (µg g <sup>-1</sup> )	R.S.D. (%)	Mean ± S.D. (µg g <sup>-1</sup> )	R.S.D. (%)	Mean ± S.D. (µg g <sup>-1</sup> )	R.S.D. (%)	Mean ± S.D. (µg g <sup>-1</sup> )	R.S.D. (%)
26 February 2003	4.91 ± 0.25	5.10	2.26 ± 0.19	8.18	2.00 ± 0.24	12.08		
3 April 2003	2.43 ± 0.46	18.95	0.35 ± 0.03	8.70	5.35 ± 0.64	12.03	2.77 ± 0.16	5.79
23 May 2003	0.16 ± 0.00	0.00	1.04 ± 0.14	13.46	4.07 ± 0.46	11.30	0.43 ± 0.01	2.33
3 July 2003	3.66 ± 0.00	0.00	3.51 ± 0.16	4.65	3.00 ± 0.43	14.43	4.51 ± 0.19	4.21
30 July 2003	1.43 ± 0.11	7.41	4.02 ± 0.92	22.80	6.07 ± 1.21	19.90	4.35 ± 0.55	12.57
3 September 2003	4.24 ± 0.29	6.79	5.98 ± 0.61	10.16	6.72 ± 0.21	3.08	3.79 ± 0.09	2.35
25 September 2003	4.35 ± 0.75	17.27	4.55 ± 0.52	11.36	8.45 ± 1.01	11.98	5.15 ± 0.36	7.00
12 November 2003	3.70 ± 0.00	0.00	2.47 ± 0.68	27.42	3.55 ± 1.12	31.38	6.03 ± 2.59	42.92
4 December 2003	3.98 ± 0.00	0.00	5.24 ± 0.93	17.82	6.56 ± 2.65	40.33	9.18 ± 0.15	1.61
11 February 2004	2.79 ± 0.00	0.00	5.96 ± 0.75	12.58	4.02 ± 0.66	16.43	3.03 ± 0.46	15.19

The relationship between mean DA concentration in hepatopancreas and size group of scallop varied from February 2003 to February 2004 (Fig. 2). The first sample on 26 February 2003, which comprised only three of the four size groups due to unavailability of very large scallops, exhibited a negative correlation with scallop size. On 3 April 2003, the second sampling date; the data exhibited a positive correlation between small, medium and large size groups. The following five samples from 23 May 2003 to 25 September 2003 inclusive showed no significant relationship and the three samples from 23 November 2003 to 11 February 2004 inclusive exhibited a positive correlation with scallop size. Inter-animal variation in DA concentration in hepatopancreas within a size group resulted in large relative standard deviations ranging from 22.7% to 82.5% (mean 39.0%) for small scallops, 15.0% to 46.4% (mean 33.6%) for medium scallops, 17.8% to 49.5% (mean 31.1%) for large scallops and 12.5% to 40.3% (mean 28.0%) for very large scallops. The results demonstrated that based on sample batches comprising 12 scallops, a number exceeding that specified in EU regulations, the shell length of scallops can influence the mean DA concentration in the sample.

Because each scallop hepatopancreas was analysed individually on each sampling occasion rather than using composites of hepatopancreas of each size group, a more detailed investigation of the relationship between DA concentration and scallop size from February 2003 to February 2004 was possible (Fig. 3). There was a highly significant negative correlation between DA concentration in hepatopancreas and shell length on 26 February 2003 ( $F_{1,32} = 77.54$ ,  $p = 0.000$ ,  $R^2 = 0.7079$ ). Highly significant positive relationships were recorded between DA concentration in hepatopancreas and shell length on 12 November 2003 ( $F_{1,46} = 38.19$ ,  $p = 0.000$ ,  $R^2 = 0.4536$ ), on 1 December 2003 ( $F_{1,46} = 24.33$ ,  $p = 0.000$ ,  $R^2 = 0.3459$ ) and on 11 February 2004 ( $F_{1,44} = 38.47$ ,  $p = 0.000$ ,  $R^2 = 0.4665$ ) though correlations were not as high during this depuration phase as during the earlier high toxin concentration phase. DA concentration in hepatopancreas exhibited similar highly significant negative and positive relationships with both total tissue weight and hepatopancreas weight on the same sample dates though correlation coefficients were lower than those between DA concentration in hepatopancreas and shell length.

Variations in DA concentration in hepatopancreas depend not only on the mass of toxin present but also on the size of the hepatopancreas itself. Consider for example a unit mass of DA taken into a large



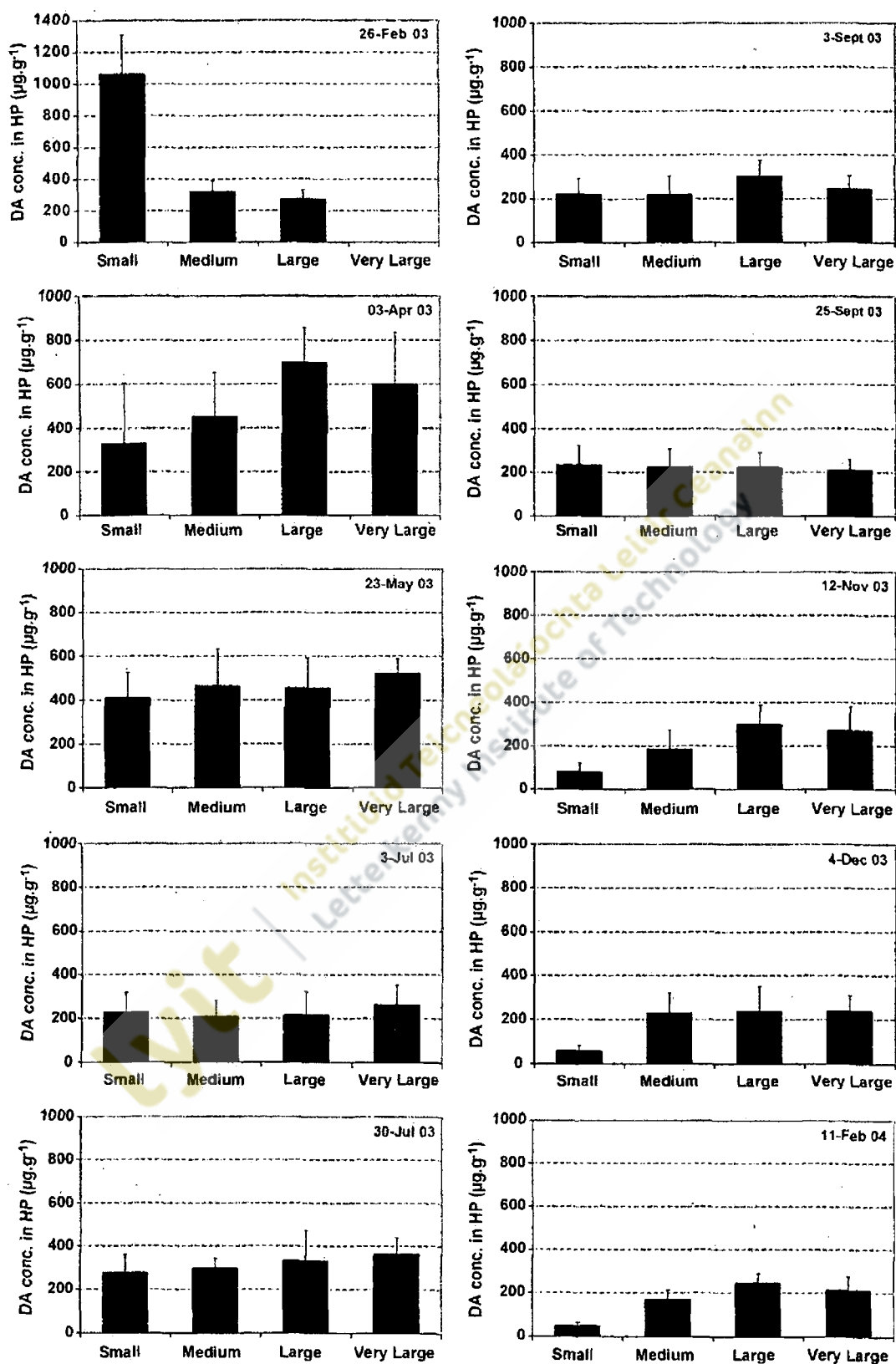


Fig. 2. Domoic acid concentration in the hepatopancreas of four size groups of king scallop, small, medium, large and very large from February 2003 to February 2004.

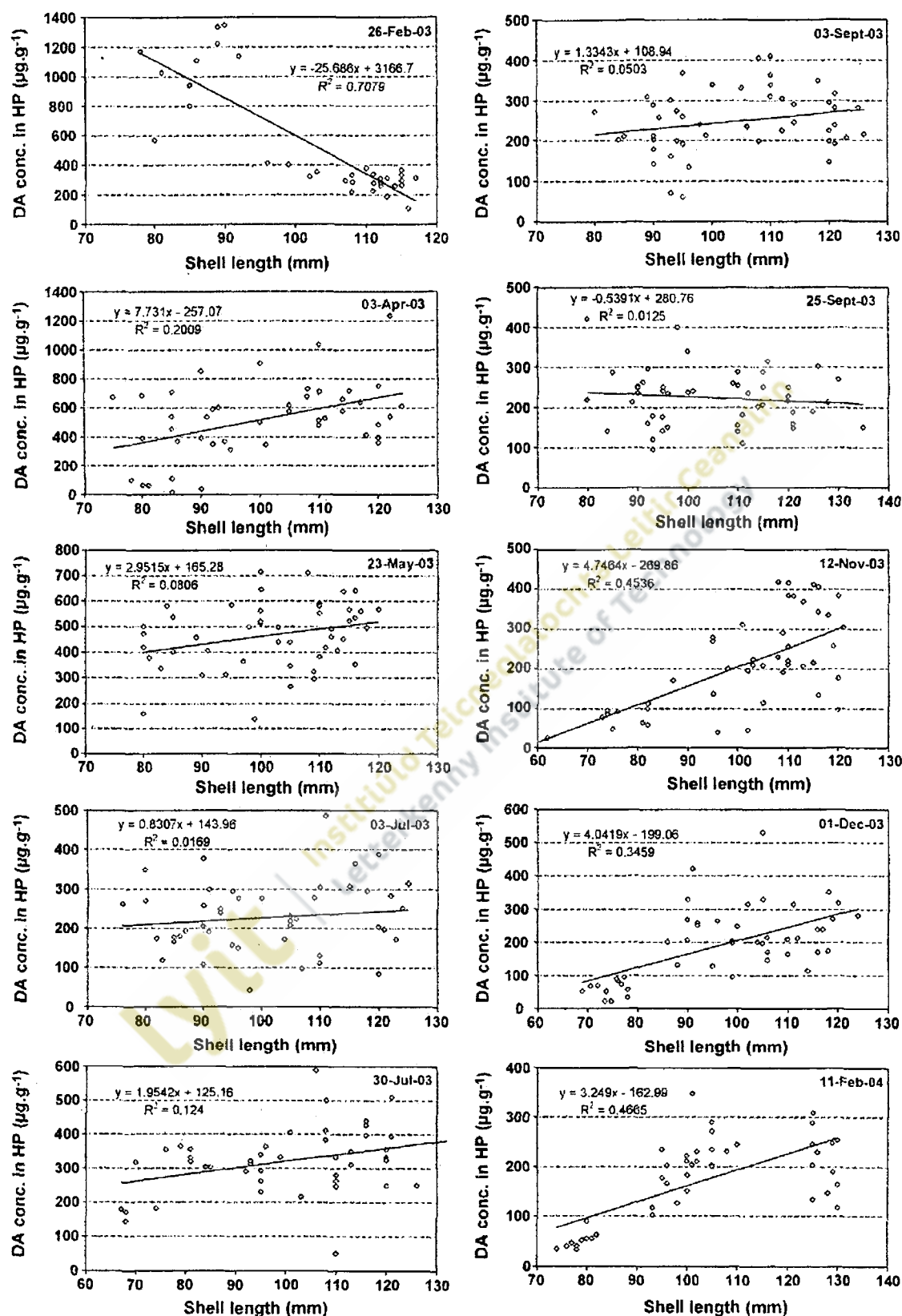


Fig. 3. The relationship between domoic acid concentration in the hepatopancreas and mean shell length of king scallops showing significant negative correlation on 26 February 2003 and weak positive correlations on 12 November 2003, 1 December 2003 and 11 February 2004.

Table 3  
Mean weights of the hepatopancreas (HP) and gonad of four size groups of king scallop from February 2003 to February 2004

	Small <sup>a</sup>		Medium <sup>a</sup>		Large <sup>a</sup>		Very large <sup>a</sup>	
	HP	Gonad	HP	Gonad	HP	Gonad	HP	Gonad
26 February 2003	2.13 ± 0.34	2.37 ± 1.01	2.67 ± 0.31	2.71 ± 1.01	2.87 ± 0.23	3.57 ± 1.03		
3 April 2003	2.50 ± 0.36	2.66 ± 0.85	3.98 ± 0.78	5.23 ± 0.89	4.44 ± 0.55	7.13 ± 1.69	5.45 ± 0.71	8.37 ± 1.23
23 May 2003	2.25 ± 0.40	6.05 ± 2.05	3.48 ± 0.48	9.49 ± 2.05	3.66 ± 0.59	12.02 ± 2.58	4.25 ± 0.31	13.15 ± 3.18
3 July 2003	2.12 ± 0.38	2.82 ± 0.53	2.53 ± 0.31	3.99 ± 0.51	3.20 ± 0.47	6.74 ± 2.19	3.77 ± 0.44	7.80 ± 2.14
30 July 2003	1.54 ± 0.35	1.18 ± 0.87	2.70 ± 0.33	2.63 ± 0.71	3.40 ± 0.44	4.02 ± 0.92	4.01 ± 0.37	4.58 ± 1.16
3 September 2003	2.58 ± 0.52	0.88 ± 0.30	3.39 ± 0.33	1.37 ± 0.43	3.82 ± 0.55	2.11 ± 0.59	5.07 ± 0.65	2.50 ± 0.51
25 September 2003	2.18 ± 0.49	0.96 ± 0.34	3.24 ± 0.33	1.15 ± 0.34	3.81 ± 0.38	1.84 ± 0.35	4.83 ± 0.54	2.39 ± 0.65
12 November 2003	1.19 ± 0.40	0.29 ± 0.21	3.35 ± 0.40	2.22 ± 0.78	3.69 ± 0.48	2.06 ± 0.66	4.03 ± 0.38	2.61 ± 0.62
4 December 2003	0.97 ± 0.20	0.15 ± 0.04	2.21 ± 0.37	1.21 ± 0.38	2.78 ± 0.39	1.95 ± 1.06	3.60 ± 0.50	2.56 ± 1.06
11 February 2004	1.13 ± 0.11	0.56 ± 0.32	2.25 ± 0.33	2.14 ± 1.10	2.66 ± 0.43	3.32 ± 0.77	4.04 ± 0.55	6.14 ± 2.57

<sup>a</sup> Mean wt. ± S.D. (g).

hepatopancreas compared to a similar mass of DA taken into a small hepatopancreas; the DA would be more diluted by the larger mass of hepatopancreas tissue than in the smaller one, with as a consequence a lower concentration being recorded in the larger hepatopancreas. During toxin depuration, the loss of a unit mass of DA from the larger hepatopancreas would have a reduced effect on the DA concentration compared to the loss of a similar unit mass of DA from the smaller hepatopancreas, which as a consequence would result in a lower concentration in the smaller hepatopancreas; the opposite effect on DA concentration to that reported during toxin accumulation. Since it is possible that differential variation in the size of the hepatopancreas could occur between scallops of different size, possibly as a result of different dietary intakes, changes in the size of the hepatopancreas in the different scallop size groups was investigated further. A similar explanation has been proposed to account for some of the variations in DA concentration in gonad, the size of this organ changing markedly during the scallop reproductive cycle. Mean weights of both hepatopancreas and gonad of the four scallop size groups on each sampling date were determined (Table 3). Mean hepatopancreas weights reflected the scallop size groups from which they were dissected and over the duration of the study exhibited significant variation, being at their largest in spring (April) and autumn (September) in all size groups. Temporal changes in DA concentration in hepatopancreas and mean weight of hepatopancreas for each size group over the study duration are illustrated in Fig. 4. On the first three sampling dates, both higher toxin concentrations and higher weights of hepatopancreas were reported indicating that changes in the weight of hepatopancreas alone are not solely responsible for changes in toxin concentration, though their

participation as a contributory factor cannot be eliminated. Minor fluctuations in DA concentration in hepatopancreas during the July 2003–November 2003 period during which toxin concentrations were more stable could be more easily explained by variations in hepatopancreas weight.

Mean gonad weights similarly reflected the scallop size groups from which they were dissected and peaked in all samples in late May prior to spawning (Slater, 2005; Bogan et al., 2006b). Temporal changes in DA concentration in gonad and mean weight of gonad for each size group over the study duration are illustrated in Fig. 5. During the gonad maturation and spawning period, increases in gonad weight, attainment of a maximum gonad size prior to spawning and decreases in weight following gamete release were matched by inverse relationships with DA concentration in gonad (Fig. 5). Fluctuations in DA concentration in gonad from late July 2003 onwards, a period during which gonad weight exhibited only minor variation, resembled more closely DA concentration in hepatopancreas over the same period.

Based on mean DA concentrations in hepatopancreas (Table 1) and gonad (Table 2) and mean weight of these organs (Table 3), the mass of DA in hepatopancreas and gonad for each size group on each sampling occasion was determined (Table 4). Consideration of the mass of DA in hepatopancreas and gonad rather than the DA concentration eliminated the effects of temporal changes in weight of these organs during the study. The mass of DA accumulated in hepatopancreas reflected the scallop size group, very large scallops accumulating more DA than smaller-sized scallops (Fig. 6), with the exception of the first sample in February 2003. A large peak in the mass of toxin in April 2003 and two smaller peaks in July 2003 and

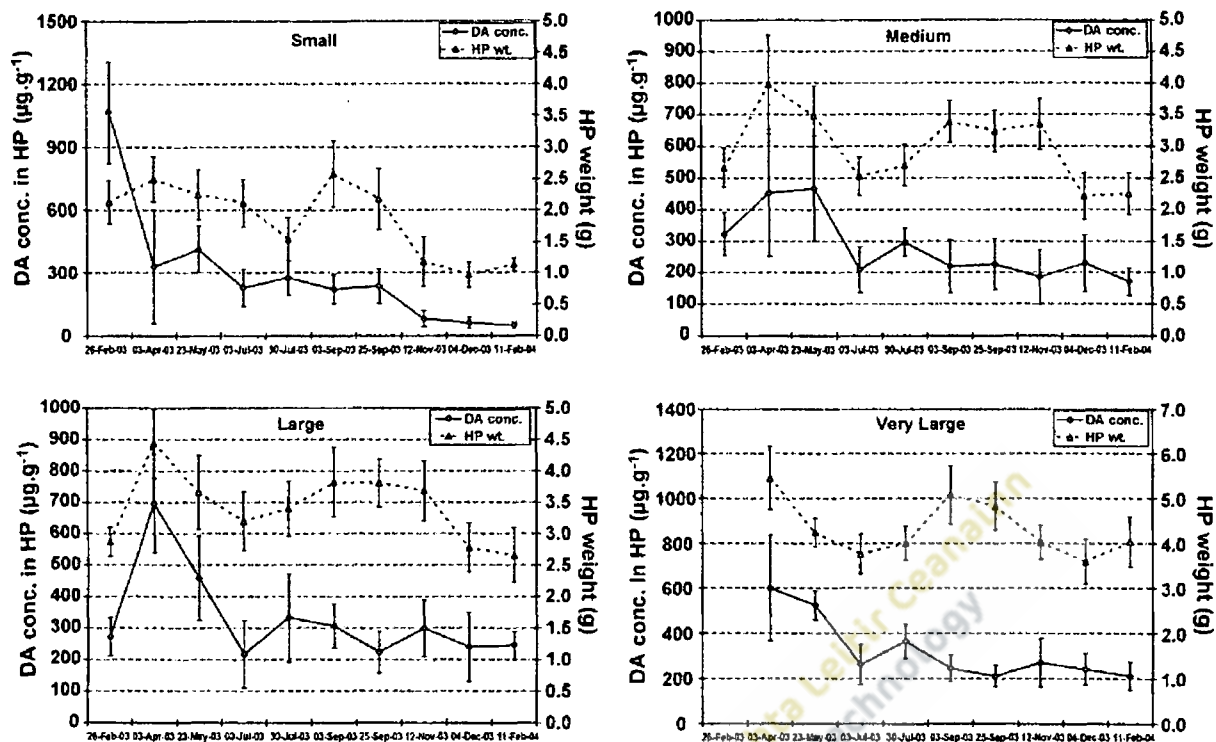


Fig. 4. The relationship between domoic acid concentration in the hepatopancreas and mean hepatopancreas weight in four size groups of king scallop from February 2003 to February 2004 (Error bars  $\pm$  standard deviation).

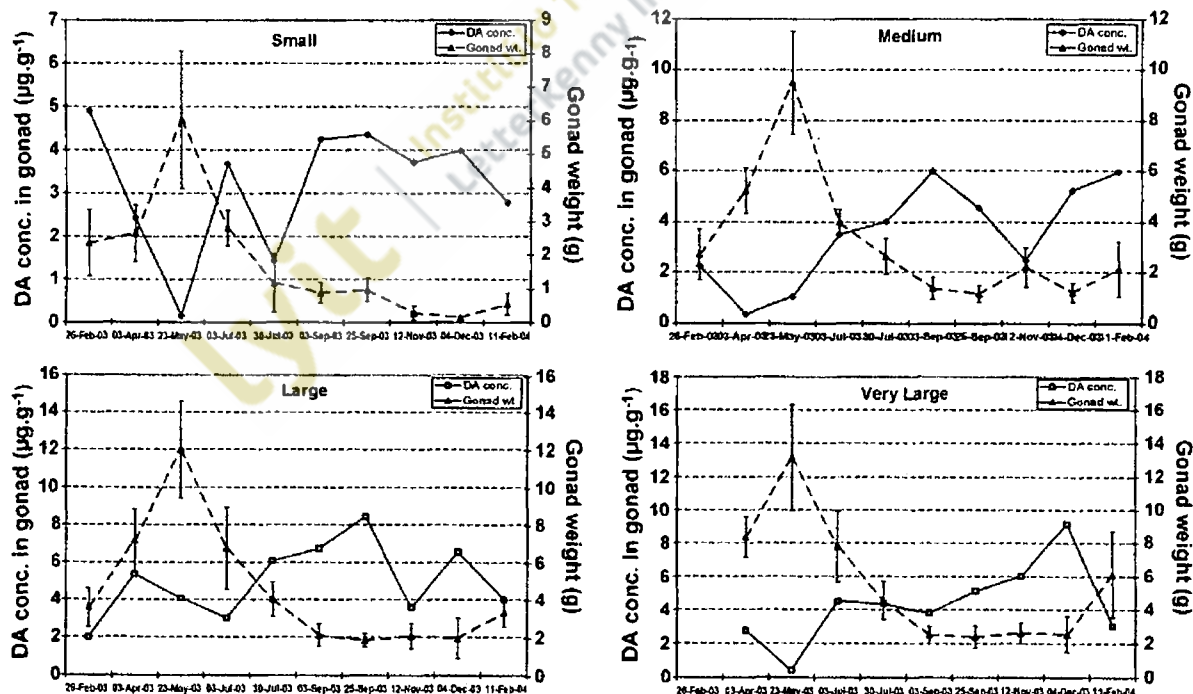


Fig. 5. The relationship between domoic acid concentration in the gonad and mean gonad weight in four size groups of king scallop from February 2003 to February 2004 (Error bars  $\pm$  standard deviation).

Table 4

Mass of DA in the hepatopancreas (HP) and gonad of four size groups of king scallop from February 2003 to February 2004

	Small <sup>a</sup>		Medium <sup>a</sup>		Large <sup>a</sup>		Very large <sup>a</sup>	
	HP	Gonad	HP	Gonad	HP	Gonad	HP	Gonad
26 February 2003	2243.3 ± 530.6	11.6 ± 5.0	852.2 ± 156.4	6.1 ± 2.3	774.5 ± 156.9	7.1 ± 2.1		
3 April 2003	820.7 ± 664.9	6.5 ± 2.1	1708.8 ± 701.5	1.8 ± 0.3	3089.7 ± 736.9	38.2 ± 9.1	3283.6 ± 1439.9	23.1 ± 3.4
23 May 2003	929.7 ± 290.2	1.0 ± 0.3	1637.1 ± 613.7	9.9 ± 2.1	1682.2 ± 584.3	49.0 ± 10.5	2229.0 ± 334.3	5.6 ± 1.4
3 July 2003	481.7 ± 204.3	10.3 ± 1.9	525.8 ± 195.4	14.0 ± 1.8	701.0 ± 380.6	20.2 ± 6.6	985.5 ± 346.3	35.1 ± 9.7
30 July 2003	442.5 ± 193.6	1.7 ± 1.2	804.7 ± 164.0	10.6 ± 2.9	1103.0 ± 413.6	24.4 ± 5.6	1455.3 ± 305.4	19.9 ± 5.0
3 September 2003	548.8 ± 161.4	3.7 ± 1.3	749.8 ± 290.1	8.2 ± 2.6	1148.0 ± 237.8	14.2 ± 4.0	1237.5 ± 293.8	9.5 ± 1.9
25 September 2003	494.8 ± 156.5	4.2 ± 1.5	750.8 ± 337.9	5.3 ± 1.5	853.6 ± 280.0	15.6 ± 3.0	1014.3 ± 251.4	12.3 ± 3.3
12 November 2003	104.2 ± 77.1	1.1 ± 0.8	610.0 ± 285.6	5.3 ± 1.9	1115.2 ± 407.3	7.3 ± 2.3	1074.2 ± 424.1	15.8 ± 3.7
4 December 2003	59.1 ± 27.8	0.6 ± 0.2	486.9 ± 144.4	6.3 ± 2.0	647.0 ± 240.7	12.8 ± 6.9	882.6 ± 313.3	23.5 ± 9.7
11 February 2004	56.2 ± 20.4	1.6 ± 0.9	384.8 ± 111.8	12.8 ± 6.6	656.7 ± 184.0	13.3 ± 3.1	842.2 ± 217.6	18.6 ± 7.8

<sup>a</sup> Mean mass DA ± S.D. (µg).

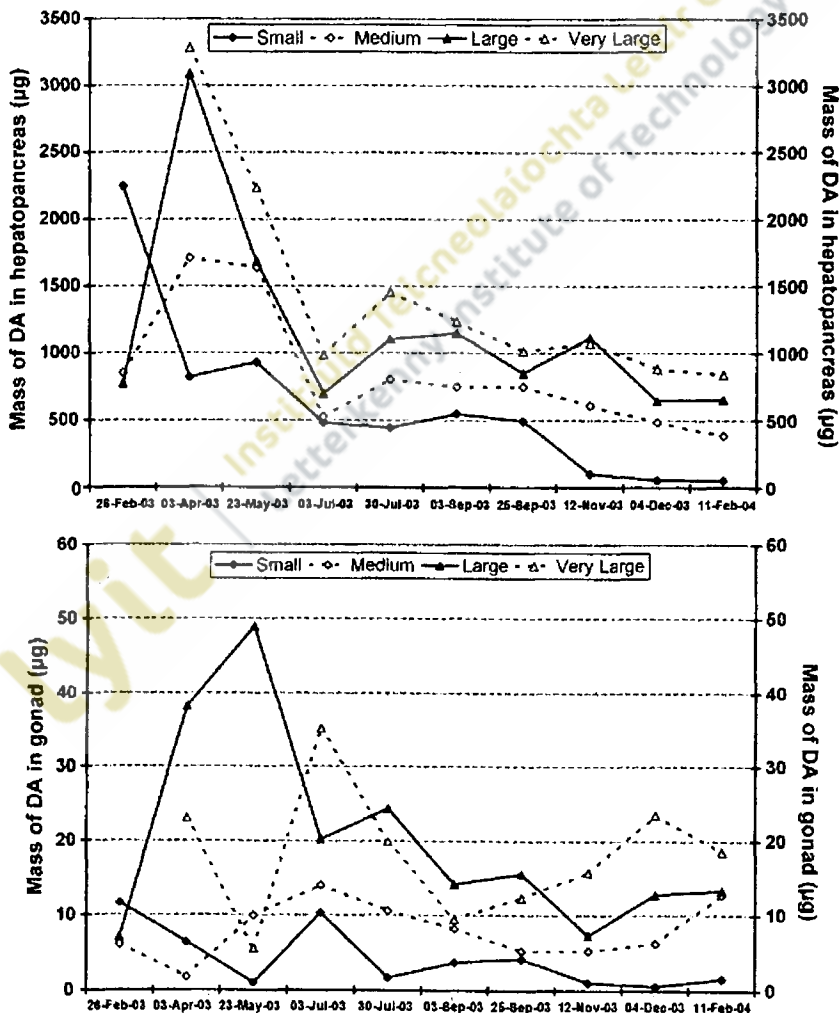


Fig. 6. The relationship between mass of domoic acid in the hepatopancreas and gonad of four size groups of king scallop from February 2003 to February 2004.



September 2003 were evident. Trends in the mass of DA in gonad in the four size groups were less evident possibly due to the more significant changes in weight of this organ during the reproductive cycle and the much lower DA concentrations reported by comparison with the hepatopancreas (Fig. 6).

#### 4. Discussion

Comparison of DA distribution between hepatopancreas, gonad and adductor muscle from the four size groups of scallop demonstrated that during the period from February 2003 to February 2004 more than 96% of toxin was always located in hepatopancreas. Similar distribution of this toxin in scallop tissues has been reported (Douglas et al., 1997; Arévalo et al., 1998; Campbell et al., 2001; Blanco et al., 2002; James et al., 2005). The highest concentration of DA recorded in hepatopancreas of an individual scallop was  $1348.1 \mu\text{g g}^{-1}$ , considerably below the highest concentrations of  $2083 \mu\text{g g}^{-1}$  reported by Arévalo et al. (1998) and  $2820 \mu\text{g g}^{-1}$  reported by James et al. (2005).

Toxin concentrations in gonad were considerably lower than in hepatopancreas and never exceeded the regulatory limit of  $20 \mu\text{g g}^{-1}$  during this study. The highest concentration of DA measured in gonad was  $9.59 \mu\text{g g}^{-1}$  much lower than values of  $69.9 \mu\text{g g}^{-1}$  reported by Arévalo et al. (1998) and  $75.47 \mu\text{g g}^{-1}$  reported by Campbell et al. (2001).

The high levels of inter-animal variability in toxin concentration recorded in all king scallop samples were similar to those quoted elsewhere (Arévalo et al., 1998; Campbell et al., 2001). The principal factor influencing toxicity is now accepted as exposure to toxic phytoplankton cells however an influence from many other factors has been suggested including seston volume (Morono et al., 2001), volume-specific toxin concentration (Morono et al., 2001), water temperature (Silvert and Subba Rao, 1992; Morono et al., 2001), water depth (Bricelj and Shumway, 1998; Curtis et al., 2000; Bogan et al., 2006a), filtration rate (Morono et al., 2001), duration of exposure (Bricelj and Shumway, 1998); animal size and body weight (Aalvik and Framstad, 1981; White et al., 1993; Arévalo et al., 1998; Bricelj and Shumway, 1998; Powell et al., 2002; Strohmeier et al., 2005; Costa et al., 2005); and reproductive condition (Cembella et al., 1994; Costa et al., 2005). Quantification of the variation in toxin concentration among individuals of the same species and from the same area and an understanding of the causes of such variation is important for the development of reliable sampling and management protocols.

Over the duration of this study, CVs of DA concentration in hepatopancreas in small-sized scallops ranged from 22.7% to 82.5% and were significantly larger than in the other three size groups; 15.0% to 46.4% in medium scallops, 17.8% to 49.5% in large scallops, 12.5% to 40.3% in very large scallops, indicative of the more variable mean DA concentration in smaller-sized scallops. The relationships between toxin concentration in hepatopancreas and shell length over the study duration demonstrated a significant negative correlation, the absence of any correlation and several significant positive correlations.

A negative correlation ( $R^2 = 0.7079$ ,  $n = 34$ ) between DA concentration in hepatopancreas and scallop shell length was reported on the first sampling occasion. Similar negative correlations have been reported from laboratory studies with mytilotoxin in mussels *M. edulis* (Aalvik and Framstad, 1981); with DA in mussels *M. edulis* (Novaczek et al., 1992); with PSP toxins in both Mediterranean mussels *Mytilus galloprovincialis* (L.) (Morono et al., 2001) and surf clams *Spisula solidissima* (Bricelj et al., 1990; Bricelj and Shumway, 1998) and from a field study of DSP toxins in *M. edulis* (Strohmeier et al., 2005). The most likely explanation for the observed negative correlation between DSP toxin concentration and shell size in *M. edulis* was considered to be faster depuration rates and hence lower concentrations in larger-sized mussels (Strohmeier et al., 2005). Given the subsequent increases in toxin concentration in all but the small scallops in the second sample in this study, it was considered that the higher DA concentrations in small scallops recorded in the first sample were due either to higher rates of toxin uptake resulting from a higher weight-specific filtration rate (Jones et al., 1992) and/or assimilation rate in smaller scallops (Morono et al., 2001), a reduced dilution effect of the smaller weight of hepatopancreas tissue resulting in a higher DA concentration or possibly a combination of both factors. To account for the differences in DA concentration reported in this study both the mass of DA in hepatopancreas and weight of the organ itself were considered. The mass of DA in hepatopancreas of small scallops was larger than that in medium and large scallops on the first sampling occasion but was reversed by the time of the second sampling occasion suggesting that differences in reported concentrations in the first sample were caused, at least in part, by a faster rate of DA uptake in small scallops. The mean weight of hepatopancreas in small scallops was less than that in the other two size groups suggesting that the higher DA concentration could have resulted from reduced

dilution of the mass of DA by hepatopancreas tissue. The relative contribution of the higher mass of DA in hepatopancreas of small scallops and the lower weight of hepatopancreas in small scallops on variation in DA concentration in hepatopancreas could not be ascertained from the results in this study. Given the scale of the differences in DA concentration between the different sizes of scallops it is considered more likely that the negative correlation between DA concentration and scallop size in the first sample was due to a combination of higher rates of uptake and/or assimilation in small scallops, though the influence of dilution of the mass of DA by the weight of hepatopancreas tissue cannot be ignored.

It might be argued that the differences observed in the first sample might have resulted from an age-dependent toxin-absorption mechanism whereby younger, small scallops held onto the toxin more efficiently than older, larger scallops. Such a mechanism might account for the variable sensitivity to toxic phytoplankton cells reported by Chavaud et al. (1998) with this scallop species and *Gymnodinium* cf. *nagasakiense* although these effects were reported with smaller 1- and 2-year-old juveniles rather than the larger scallops used in this study. Subsequent increases in toxin concentration in the second sample suggested that the differences in DA concentration between the size groups recorded in the first sample had not resulted from faster depuration in larger scallops.

No correlation between DA concentration in hepatopancreas and scallop shell length was exhibited by data from individual hepatopancreas analysis on six subsequent sampling occasions from 3 April to 25 September ( $R^2$  ranged from 0.0125 to 0.2009,  $n = 46$ , 47). Similar results were reported in king scallop of 8–10 cm from the Ria de Arousa ( $R^2 = 0.040$ ,  $n = 72$ ) and from the Rias of Muros-Noia, Arousa and Vigo ( $R^2 = 0.038$ ,  $n = 61$ ) (Arévalo et al., 1998). There was no relationship between DA concentration and body size in the sand crab *Emerita analoga* (Powell et al., 2002) or the common cuttlefish *Sepia officinalis* (Costa et al., 2005). With PSP toxins, geoduck clams were reported to have no relationship with clam weight over a 5-month sampling period (Curtis et al., 2000). Toxin concentration during the period in which no correlation with shell length was observed exhibited wide inter-animal variation irrespective of scallop size.

Significant but weak, positive correlations between DA concentration in hepatopancreas and shell length were reported on each of the last three sample dates ( $R^2 = 0.4536$ ,  $n = 48$ ;  $R^2 = 0.3459$ ,  $n = 48$ ;  $R^2 = 0.4665$ ,  $n = 46$ ). The most likely explanation for these positive

correlations was considered to be faster depuration rates in smaller-sized scallops. In support of this explanation, comparative studies between different sizes of mussels *M. edulis* have reported faster rates of DA depuration in smaller mussels (Novaczek et al., 1992). Earlier studies of metabolic rates in mussels of different sizes reported faster metabolic rates in smaller individuals (Hawkins et al., 1990; Widdows et al., 1979). As with the first sampling occasion, to account for differences in DA concentration in hepatopancreas between size groups, both mass of DA in hepatopancreas and weight of hepatopancreas itself over the last three sampling occasions were considered. The mass of DA in hepatopancreas of the four different size groups of scallop reflected their shell lengths; small, medium, large and very large scallops having a relatively higher mass of DA present in hepatopancreas. Small-sized scallops exhibited a rapid decline in mass of DA between the seventh and eighth samples and remained at a low level on the remaining three sampling occasions, whereas mass of DA in the other three size groups exhibited a more gradual decline over this period. The mean weight of hepatopancreas in small and very large size groups of scallop was relatively constant in the last three samples, having declined considerably during the two preceding sampling occasions, whereas in medium and large scallop size groups it declined markedly between the eighth and ninth sampling occasion, losing 34% and 25% in weight respectively, without a corresponding increase in DA concentration. The relative contribution to variations in the decline of DA concentration between different scallop size groups of mass of DA in hepatopancreas and weight of hepatopancreas could not be ascertained from results in this study. Clearly depuration of this toxin from *P. maximus* was a slow process and has been reported to be one of the slowest among bivalve species studied (Blanco et al., 2002). Since the trend in both the concentration and mass of DA were broadly similar it was considered that the positive correlations reported in the last three samples was more closely related to the rate of DA depuration than with changes in the weight of hepatopancreas, though the influence of the latter cannot be ignored.

By comparison with DA concentrations in hepatopancreas, toxin concentrations in gonad were always below the regulatory limit of  $20 \mu\text{g g}^{-1}$  and never exceeded  $10 \mu\text{g g}^{-1}$ . These lower DA concentrations, the increased significance of analytical error associated with measurement of low DA concentrations and possibly high inter-animal variability reported in the literature, presented some difficulties in understanding the relation-



ship between toxin concentration and scallop size based on this tissue. Comparison of DA concentrations and gonad weights from the samples between April and early July clearly exhibited an inverse relationship, low DA concentrations being associated with large gonad weight, demonstrating the significant dilution effect of this tissue on toxin concentration. The trends in DA concentration in gonad in the different size groups of scallop following the decline in gonad weight due to spawning were not dissimilar to those exhibited by the hepatopancreas though given the low concentrations present statistical correlation was not attempted. Similar difficulties to those encountered in this study in interpreting changes in DA concentration in gonad have been reported elsewhere (FSA, 2001; Bogan et al., 2006b). Understanding the variation in the mass of DA in gonad from the four size groups, unlike the mass of DA in hepatopancreas, was also more difficult and was attributed to the low concentrations present, the high inter-animal variation, the increased significance of analytical error and the small weight of the gonads in some scallops, particularly those in the smaller-size group.

#### 4.1. Significance of the findings

Previous studies reporting no relationship between the DA concentration and the shell length of king scallop have been based on samples collected on a single occasion (Arévalo et al., 1998; Campbell et al., 2001). By contrast, the results from this study based on determination of DA concentration in hepatopancreas in a wider range of scallop shell lengths, as suggested by Campbell et al. (2001), on 10 sampling occasions demonstrated that the shell length of scallops could influence the DA concentration. Smaller scallops exhibited higher DA concentrations during toxin uptake and lower DA concentrations during toxin depuration resulting in negative and positive correlations between DA concentration and scallop shell length, respectively. The implications of such findings from a toxin testing perspective would initially suggest that scallop shell length should be taken into account during the design and implementation of sampling protocols for DA concentration in king scallops. However, EU Commission Decision 2002/226 refers to the testing of harvestable product, implying that the scallops for testing should be above the legal landing size of 100 mm shell length. Re-examination of data from those sampling occasions exhibiting a negative or positive correlation, using only scallops greater than or equal to 100 mm, resulted in the absence of any correlation between DA concentration and scallop shell length. Data presenting a negative

correlation ( $R^2 = 0.7079$ ) on the first sampling occasion exhibited no correlation ( $R^2 = 0.0769$ ,  $n = 22$ ) using only scallops greater than or equal to 100 mm. Similarly data presenting weak positive correlations from the last three sampling occasions ( $R^2 = 0.4536$ ,  $0.3459$ ,  $0.4665$ ) exhibited no correlations ( $R^2 = 0.0339$ ,  $n = 31$ ;  $R^2 = 0.0014$ ,  $n = 24$ ;  $R^2 = 0.0232$ ,  $n = 27$ ) using only scallops greater than or equal to 100 mm. Hence for the development of reliable sampling protocols only scallops larger than the minimum commercial size of 100 mm or edible parts from scallops larger than the minimum commercial size should be used for toxin testing.

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## Appendix 2. Paper 2

Spatial variability of domoic acid concentration in king scallops  
*Pecten maximus* off the southeast coast of Ireland. Harmful Algae.

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P. Hess. 2006.

### Author Contributions

Y.M.B., K.B. and P.H. designed the research programme.

A.H. collected the scallops.

Y.M.B., K.B. and A.H. dissected the scallops.

Y.M.B. performed the analysis.

Y.M.B., J.W.S. and D.J.K. analyzed the data.

Y.M.B., A.H., J.W.S. and P.H. prepared the manuscript.

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## Spatial variability of domoic acid concentration in king scallops *Pecten maximus* off the southeast coast of Ireland

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### Abstract

Amnesic shellfish poisoning (ASP) has proved problematic in Irish king scallop, *Pecten maximus* fisheries necessitating restrictions on the sale of fresh scallops (in-shell), which achieve a higher market price than frozen processed product. An investigation of variability in domoic acid (DA) concentration in king scallop from 69 sampling sites within a limited area off the southeast coast of Ireland was performed. Variation in DA concentration was examined in the whole area, within smaller sub-areas, with size, age, and water depth. Mean DA concentrations in whole scallop ranged from 6.5 to 154.3  $\mu\text{g g}^{-1}$ , with an overall mean of  $40.6 \pm 30.8 \mu\text{g g}^{-1}$ . The concentration in gonad exceeded 20  $\mu\text{g g}^{-1}$  in 17 sites and in adductor muscle in 3 sites. Significant differences in DA concentration were detected between scallops from different sampling stations. Whole scallop tissue and individual scallop tissues with the exception of gonad, exhibited significant negative correlations with water depth. Highest DA concentrations were recorded in inshore, shallow sites and lowest DA concentrations in deeper offshore waters. Significant positive correlations between DA concentration in hepatopancreas and scallop size were exhibited at inshore sites but not at offshore sites. High inter-animal and spatial variability in toxin concentration demonstrated the importance of a reliable sampling protocol for the management of ASP outbreaks to ensure public safety and to avoid unnecessary fishery closures.

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**Keywords:** Amnesic shellfish poisoning (ASP); Domoic acid; *Pecten maximus*; Scallop; Spatial variation

### 1. Introduction

Production of domoic acid (DA), a small tricarboxylic amino acid belonging to the kainoid class of compounds, has been reported in ten species of the diatom genus *Pseudo-nitzschia* (Bates, 2000). This

water-soluble neurotoxin, existing in ten isomeric forms, can accumulate in bivalves such as scallops and mussels filter-feeding on these toxic phytoplankton species (Zaman et al., 1997). Consumption of shellfish contaminated with DA can cause amnesic shellfish poisoning (ASP), the early symptoms of which include nausea, gastroenteritis and vomiting followed within 48 h by neurological symptoms such as confusion, lethargy, disorientation and memory loss that can persist indefinitely (Quilliam and Wright, 1989; Todd, 1993). ASP was first recorded in Canada in 1987, when over

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one hundred people became ill and three people died after consuming mussels *Mytilus edulis* contaminated with DA.

In Europe the detection of DA in cultured mussels from Galicia, Spain in 1994 (Arévalo et al., 1997) and in a range of Portuguese shellfish in 1995 (Vale and Sampayo, 2001) led to amendment 61/97 of EU Directive 91/492/EEC which established the maximum allowable concentration of DA in whole shellfish or edible parts as  $20 \mu\text{g g}^{-1}$ . Extensive shellfishery closures have since been recorded in European waters, perhaps the most significant being those applied to the king scallop fishery off the west coast of Scotland from 1999 onwards (Gallacher et al., 2001) resulting in considerable financial hardship for scallop fishermen (Smith et al., 2006). Localisation of the majority of toxin in the scallop hepatopancreas, slow depuration rates leading to extended periods of closure and high inter-animal variation between scallops led to introduction of EU Commission Decision 2002/226/EC which allowed harvesting of scallops with a whole body DA concentration exceeding  $20 \mu\text{g g}^{-1}$  if the parts to be marketed, principally the adductor muscle and gonad, contained less than  $4.6 \mu\text{g g}^{-1}$ . Implementation of Commission Decision 2002/226/EC in some countries has been based on area management whereby determination as to whether an ICES box or area remains open or closed for fishing is based on DA concentration from a single sample of 10 scallops. High inter-animal variability in DA concentration and concern regarding the lack of understanding of the causes of such variation in scallops has been widely reported (Arévalo et al., 1998; Campbell et al., 2001).

In Ireland this toxin has similarly proven particularly problematic in king scallop *Pecten maximus* fisheries and in late 1999 and early 2000 elevated DA concentrations led to short-term prohibition of all scallop harvesting. The scheme currently used for management of this biotoxin in Ireland distinguishes between scallops produced by aquaculture and those from the wild fishery. Cultivated scallops are sampled weekly from defined sampling points in relevant aquaculture bays and managed according to the DA limits prescribed in legislation. Samples must be provided for testing 2 weeks prior to the commencement of harvesting and must be approved as being suitable for entry into the marketplace. In the event of high DA concentrations, harvesting of cultivated scallops from aquaculture sites can easily be postponed until levels are below  $20 \mu\text{g g}^{-1}$ . This flexibility in the time of harvest to correspond with DA concentrations below that prescribed in legislation

has allowed the marketing of cultivated scallops on the fresh in-shell market where premium prices can be attained. The biotoxin management scheme applied to scallops from the wild fishery necessitates that all the catch be shucked to remove the hepatopancreas and then frozen prior to testing. No product is allowed to enter the marketplace before test results indicate that the DA concentration in adductor and gonad is below  $20 \mu\text{g g}^{-1}$ . This landings-based approach to scallop fishery management has allowed resumption of harvesting from fishing grounds that otherwise would have remained closed under amendment 61/97 of EU Directive 91/492/EEC, but has necessitated that all the catch be processed prior to sale. This distinction in biotoxin management between cultivated and wild scallops has caused some concern amongst fishermen. Lack of access to the high value fresh market for wild fishery scallops has impacted in particular in those regions off the southeast coast of Ireland in which scallop fisheries expanded in scale and importance during the 1990s. Scallop beds in this region are larger than in other locations around the Irish coast and the fleet, based in Kilmore Quay and Duncannon, Co., Wexford, currently accounts for 90% of Ireland's scallop landings (Tully et al., 2002), which exceeded 1700 tonnes in 2004 (BIM, 2005). This investigation of spatial variation in DA concentration in a limited fishing area was prompted by an interest in examination of the scientific basis for a possible re-establishment of the market for fresh, in-shell scallops. All samples were procured from an area less than  $0.5^\circ$  latitude by  $1^\circ$  longitude, the size of an ICES fishing box, and the effect of both water depth and scallop size on variation of DA concentration was investigated.

## 2. Materials and methods

### 2.1. Sample procurement

King scallops were dredged from 69 sampling stations in the Mine Head and Saltees grounds off the southeast coast of Ireland in September/October 2003 as part of a fishery stock assessment by Bord Iascaigh Mhara (BIM) (Irish Sea Fisheries Board). GIS coordinates at the start and end of each haul together with mean water depth over the duration of each sampling were recorded. All sampling stations were located within an area from  $51.55^\circ\text{N}$  to  $52.05^\circ\text{N}$  and  $6.60^\circ\text{W}$  to  $7.45^\circ\text{W}$  (Fig. 1), less than the area of an ICES box ( $0.5^\circ$  latitude by  $1^\circ$  longitude) currently used in some shellfish monitoring programmes. All scallop samples were bagged and stored

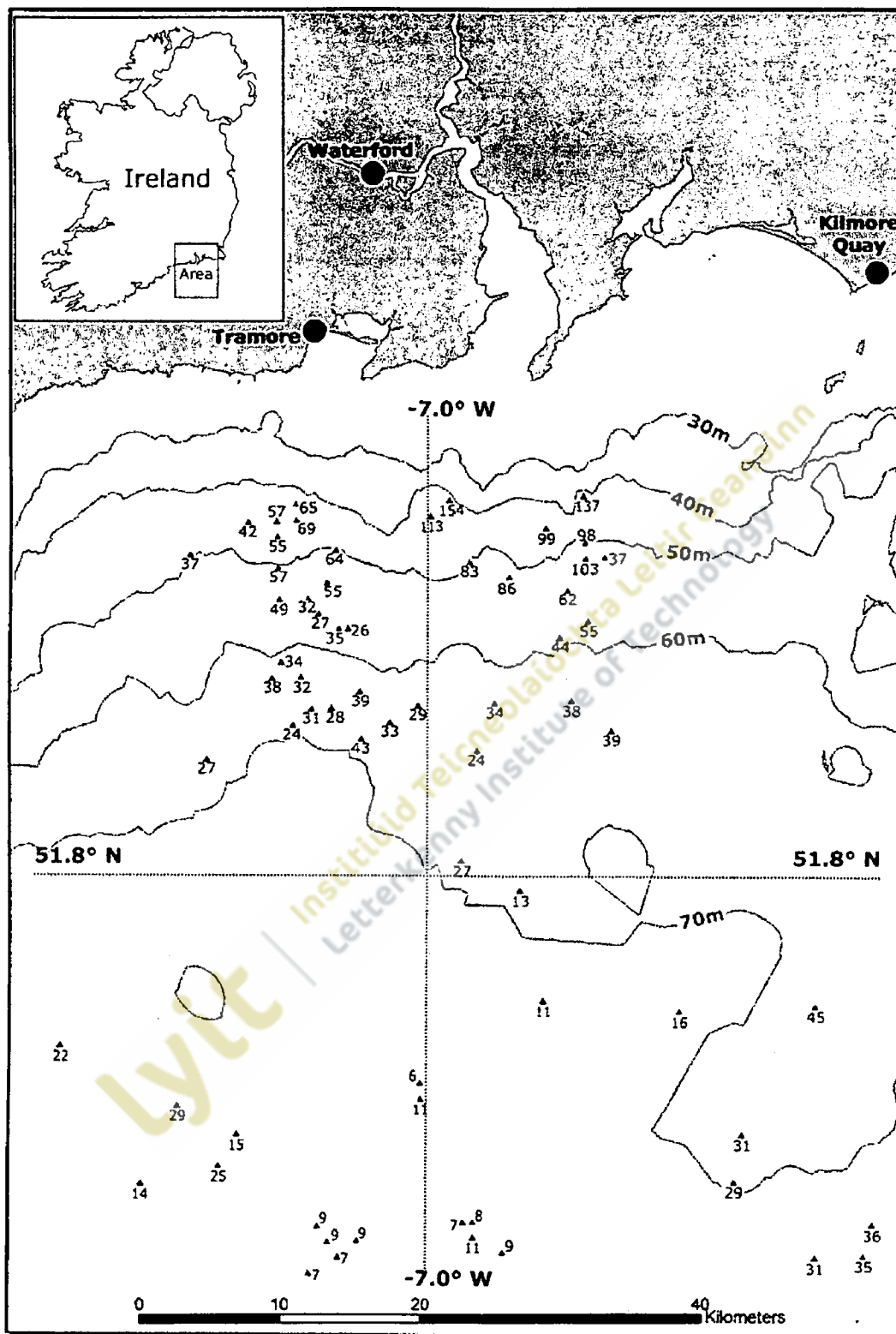


Fig. 1. DA concentration in  $\mu\text{g g}^{-1}$  in whole scallop *Pecten maximus* at 69 sampling stations off the southeast coast of Ireland.



in a commercial freezer at  $-20^{\circ}\text{C}$  in Kilmore Quay, Co., Wexford.

## 2.2. Spatial variability in DA concentration

To investigate variability in DA concentration within the fishing ground, 12 scallops, where available, of commercial size (100–110 mm) were selected randomly from each station and the shell dimensions and age using ring analysis determined for each. Scallops ( $n = 782$ ) were dissected and the total tissue weight and individual weight of the hepatopancreas, gonad, adductor muscle and tissue remainder recorded. Composite samples of each tissue; hepatopancreas, gonad, adductor and tissue remainder from each station were prepared for analysis.

## 2.3. Variation in DA concentration with scallop size

Variations in DA concentration with scallop size were investigated at five of the stations selected on the basis of area coverage and scallop abundance (Fig. 2). Sub-samples of 12 scallops, where available, were randomly selected from each station in each of the following shell length groups: small (70–85 mm), medium (85–100 mm), large (100–115 mm) and very large ( $>115$  mm) and shell dimensions (length, height and depth) and age of each scallop recorded. Scallops ( $n = 217$ ) were dissected and the total tissue weight and individual weight of the hepatopancreas, gonad, adductor muscle and tissue remainder recorded. The hepatopancreas of each scallop from each size group at each station was analysed individually. Composite samples of pooled gonad, pooled adductor muscle and pooled tissue remainder from each size group at each station were prepared for analysis.

## 2.4. Domoic acid extraction and analysis

DA was extracted from scallop tissue samples using procedures based on Quilliam et al. (1995) with the addition of a double extraction procedure (Harkin et al., 2004). Extraction was performed from approximately  $4.0 \pm 1.0$  g of tissue homogenate with 16 ml of 50:50 extraction solvent (methanol:water) in a blender (Ultra Turrax T25, IKA®-Works.) for 1 min at high speed. The extract was centrifuged at 4500 rpm for 15 min and the supernatant decanted into a 50 ml flask. The pellet was re-extracted with a further 16 ml of extraction solvent as above and the supernatants combined. The combined supernatant was made up to 50 ml with extraction solvent. A sample of the combined supernatant was

filtered using a methanol-compatible  $0.25\text{ }\mu\text{m}$  syringe filter and the combined concentration of DA and *epi*-DA in the filtered extract determined using a Shimadzu HPLC/DAD (Mason Technology, Dublin). In those instances where the combined concentration exceeded the concentration of the highest standard, the filtered extract was diluted with extraction solvent and the measurement of DA and *epi*-DA repeated. DA and *epi*-DA were identified from their retention times and UV spectra. Calibration was performed externally using six DA calibration standards between  $0.2$  and  $10\text{ }\mu\text{g ml}^{-1}$  prepared from certified reference standard obtained from the NRC, Canada. Calibration curves were prepared daily and were always linear ( $R^2 > 0.999$ ) with a QC standard run every 12th sample. Quality assurance of all data were provided by certified reference material (MUS-1B) obtained from the NRC, Canada and in-house Laboratory Reference Material (LRM) ( $37.0\text{ }\mu\text{g g}^{-1}$ ) prepared from king scallop tissue by the Marine Institute. Analysis of the LRM exhibited a CV of 6.4% ( $n = 20$ ) over the duration of the investigation. All solvents used were HPLC grade and supplied by Labscan, Dublin.

Statistical analysis of all data were performed using SPSS, Version 12.

## 3. Results

### 3.1. Spatial variability in DA concentration

#### 3.1.1. Variation with location

The mean, standard deviation, coefficient of variation (CV) and range of DA concentrations in whole scallop, hepatopancreas, gonad, adductor muscle and tissue remainder from the sampling area are summarised in Table 1. Concentrations of DA in whole scallop tissue ranged from  $6.5\text{ }\mu\text{g g}^{-1}$  to a maximum of  $154.3\text{ }\mu\text{g g}^{-1}$ . DA concentration in gonad and adductor muscle exceeded the regulatory limit of  $20\text{ }\mu\text{g g}^{-1}$  at 17 stations and 3 stations, respectively. DA concentrations in adductor muscle were typically one or two orders of magnitude below that of hepatopancreas. CVs of DA concentration in different tissues ranged from 71.3% to 106.2%.

DA concentrations in whole scallop and scallop tissues from the 69 sampling stations within an area of  $55.7\text{ km} \times 58.5\text{ km}$  are provided in Table 2. DA concentration in whole scallop exhibited a strong correlation with DA concentration in hepatopancreas ( $F_{1,67} = 1284.3$ ,  $p = 0.000$ ,  $r = 0.98$ ) but a weak correlation with DA concentration in gonad ( $F_{1,67} = 18.01$ ,  $p = 0.000$ ,  $r = 0.46$ ).

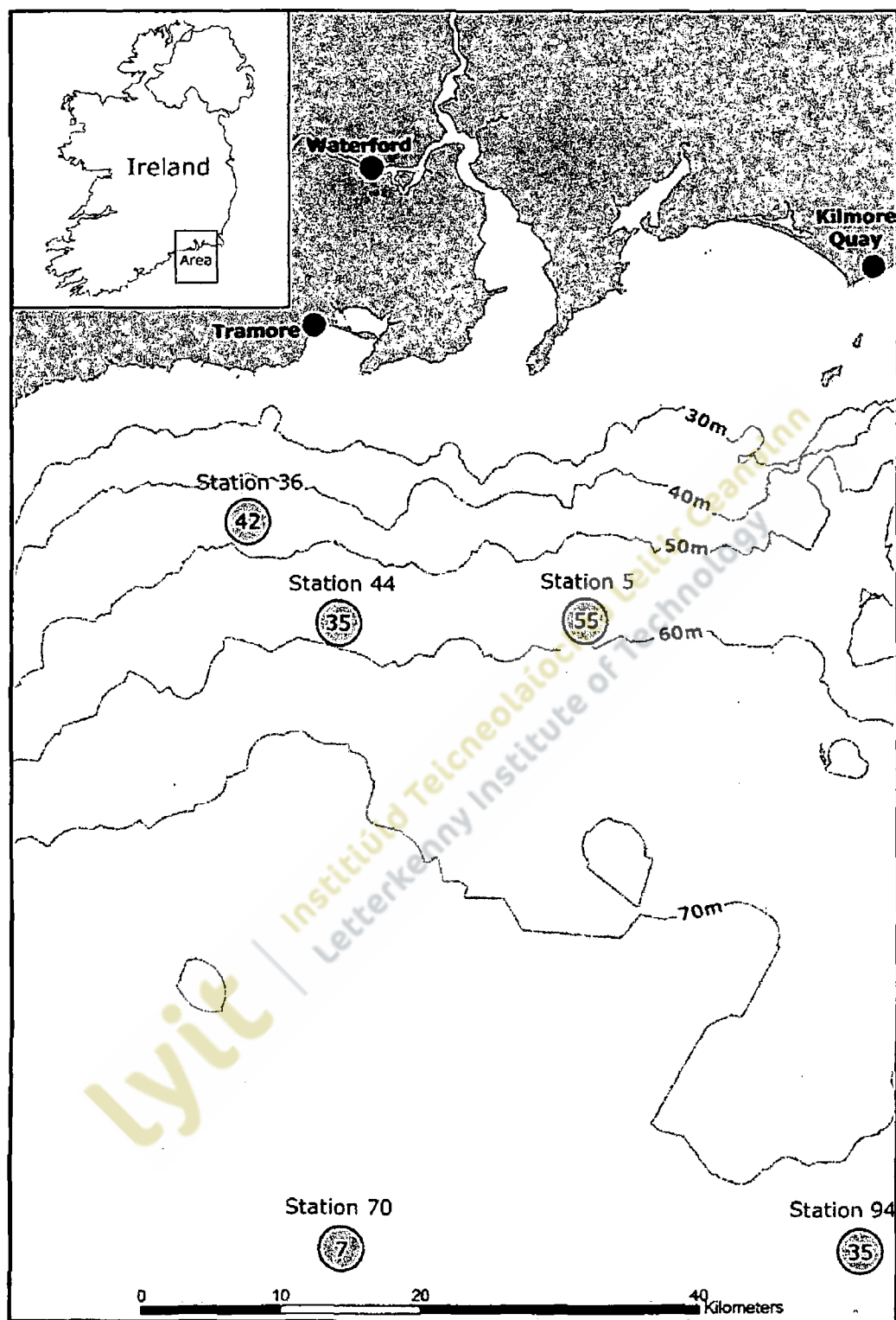


Fig. 2. Location of sampling stations off the southeast coast of Ireland used in the study of variation in DA concentration with scallop size. Figures inside the markers indicate DA concentration in  $\mu\text{g g}^{-1}$  in whole scallop *P. maximus* at each station.

Table 1  
Summary of DA concentration in whole scallop *Pecten maximus* and individual tissues from 69 stations in the sampling area

	Whole scallop	Hepatopancreas	Gonad	Adductor muscle	Remainder
Mean DA conc. ( $\mu\text{g g}^{-1}$ )	40.6	345.7	15.1	5.4	15.7
S.D. ( $\mu\text{g g}^{-1}$ )	30.8	299.6	10.8	5.7	14.1
%CV	75.9	86.7	71.3	106.2	89.7
Range ( $\mu\text{g g}^{-1}$ )	6.5–154.3	44.7–1531.0	2.0–61.3	0.2–31.8	0.2–68.4

Table 2  
Concentration of DA in whole scallop *Pecten maximus* and individual tissues from 69 sampling stations and hypothetical status of the fishery if EU Commission Decision 2002/226 were applied

Station ID	n	Mean domoic acid concentration ( $\mu\text{g g}^{-1}$ )				Status of fishery
		Whole scallop	Hepatopancreas	Gonad	Adductor	
1	9	98.3	762.8	29.9	13.2	Closed
2	8	103.2	1017.6	27.8	10.4	Closed
3	6	37.0	375.2	16.2	12.1	Closed
4	11	62.4	524.6	20.7	9.7	Closed
5	12	55.1	478.4	15.7	8.7	Closed
6	12	44.1	342.2	14.3	3.0	Restricted harvest roe-off
11	12	38.8	284.1	33.7	3.6	Restricted harvest roe-off
13	12	34.0	285.2	7.7	2.6	Restricted harvest roe-off
15	12	29.1	239.7	8.8	2.0	Restricted harvest roe-off
19	7	136.6	1530.6	35.4	15.4	Closed
20	5	99.2	1035.6	17.1	4.5	Restricted harvest roe-off
21	12	70.3	674.3	19.6	4.4	Restricted harvest roe-off
22	11	86.1	672.6	15.8	5.3	Closed
23	12	82.8	925.9	16.7	9.7	Closed
25	12	113.0	1023.9	24.8	26.1	Closed
26	10	154.3	1257.2	29.2	31.8	Closed
27	12	64.1	528.5	13.2	8.7	Closed
28	12	54.6	485.5	28.0	4.2	Restricted harvest roe-off
30	12	56.9	475.4	16.7	5.0	Closed
31	12	65.0	669.2	18.5	4.3	Restricted harvest roe-off
32	12	56.7	367.6	10.1	8.7	Closed
33	12	68.7	555.9	12.2	9.0	Closed
34	12	55.3	299.2	16.4	21.8	Closed
36	12	41.6	230.8	22.2	8.5	Closed
37	12	36.8	296.4	7.2	1.7	Restricted harvest roe-off
39	10	48.6	380.8	17.2	10.4	Closed
40	11	32.0	269.3	17.5	2.3	Restricted harvest roe-off
42	12	27.1	205.6	8.3	5.3	Closed
43	12	25.7	229.7	5.0	3.4	Restricted harvest roe-off
44	12	34.5	286.7	19.7	4.2	Restricted harvest roe-off
45	12	27.3	252.4	5.8	2.5	Restricted harvest roe-off
46	12	33.0	224.4	29.6	5.0	Closed
47	12	43.0	372.1	8.1	3.1	Restricted harvest roe-off
48	12	38.8	335.7	5.1	3.7	Restricted harvest roe-off
49	12	27.8	224.5	61.3	5.8	Closed
50	12	32.3	259.7	6.2	3.4	Restricted harvest roe-off
51	12	38.0	287.3	19.6	2.5	Restricted harvest roe-off
53	12	33.5	280.0	7.0	4.2	Restricted harvest roe-off
55	12	26.9	251.0	3.2	2.1	Restricted harvest roe-on
56	12	24.4	210.0	10.9	2.4	Restricted harvest roe-off
57	12	31.2	244.7	3.5	3.1	Restricted harvest roe-on
58	12	37.6	309.0	7.1	5.9	Closed
59	12	23.8	193.9	2.6	2.4	Restricted harvest roe-on
62	12	22.4	170.6	8.0	2.0	Restricted harvest roe-off

Table 2 (Continued)

Station ID	n	Mean domoic acid concentration ( $\mu\text{g g}^{-1}$ )				Status of fishery
		Whole scallop	Hepatopancreas	Gonad	Adductor	
64	12	28.8	208.8	4.1	2.9	Restricted harvest roe-on
65	7	15.3	130.2	12.9	2.3	Open
66	12	25.4	192.3	16.8	2.1	Restricted harvest roe-off
67	12	14.3	107.7	6.3	1.0	Open
69	12	7.3	62.1	6.3	1.0	Open
70	12	7.0	53.4	5.3	0.8	Open
71	12	9.2	55.6	13.1	0.6	Open
72	12	9.5	74.2	7.9	0.5	Open
73	12	9.0	63.7	9.2	1.1	Open
76	12	6.5	85.8	2.0	0.4	Open
78	12	10.9	74.3	3.7	0.8	Open
79	12	7.5	62.5	3.0	0.2	Open
80	7	10.9	44.7	2.1	4.1	Open
81	12	8.3	53.4	2.2	0.3	Open
82	12	8.6	65.3	9.8	0.6	Open
84	8	13.1	101.8	10.0	1.3	Open
85	12	11.0	95.9	6.8	1.8	Open
86	12	15.9	118.2	15.1	2.6	Open
88	12	44.9	386.1	14.1	3.9	Restricted harvest roe-off
90	12	30.6	244.9	32.6	8.2	Closed
92	12	31.3	225.0	27.1	6.2	Closed
94	12	35.1	270.2	30.2	5.5	Closed
96	12	35.9	273.0	32.8	5.7	Closed
97	12	28.6	248.1	25.1	4.2	Restricted harvest roe-off
99	12	27.1	229.7	21.9	4.2	Restricted harvest roe-off

DA concentrations in whole scallop were categorised into four groups: 0–20, 20–50, 50–80 and  $>80\text{ }\mu\text{g g}^{-1}$  and the location and characteristics of the scallops from each group examined (Table 3). Sampling stations in the

high DA concentration group ( $>80\text{ }\mu\text{g g}^{-1}$ ) were located in inshore waters directly opposite Waterford Harbour while those in the low DA concentration group ( $0\text{--}20\text{ }\mu\text{g g}^{-1}$ ) were located in offshore waters (Fig. 1).

Table 3  
Characteristics of scallop *Pecten maximus* from the four DA concentration groups

Conc. group	Shell length (mm)	Shell height (mm)	Age (year)	Total tissue weight (g)	Gonad weight (g)	Adductor weight (g)	HP weight (g)	DA conc. in whole scallop ( $\mu\text{g g}^{-1}$ )	Water depth (m)
0–20 $\mu\text{g g}^{-1}$									
Mean	109.0	99.5	5.0	40.7	2.2	19.5	4.2	10.3	73.0
S.D.	4.5	3.7	0.8	7.8	1.8	4.2	0.9	3.0	1.6
CV	4.1	3.7	15.1	19.2	80.4	21.6	21.2	28.2	2.2
20–50 $\mu\text{g g}^{-1}$									
Mean	109.3	99.7	4.9	43.8	4.0	21.2	4.0	33.1	64.1
S.D.	4.2	4.0	0.7	8.6	3.1	4.6	0.5	6.5	7.4
CV	3.8	4.0	15.3	19.6	78.4	21.8	13.0	16.2	10.4
50–80 $\mu\text{g g}^{-1}$									
Mean	109.4	97.6	4.9	47.5	5.4	22.3	4.3	60.9	50.3
S.D.	4.2	4.1	0.7	7.8	3.5	4.3	1.0	5.9	4.5
CV	3.8	4.2	14.4	16.3	65.1	19.4	21.9	9.7	8.9
$>80\text{ }\mu\text{g g}^{-1}$									
Mean	109.1	98.2	4.7	50.6	7.4	23.5	4.1	109.2	50.1
S.D.	4.8	4.8	0.8	9.3	3.4	4.4	0.7	24.7	4.6
CV	4.4	4.8	17.1	18.3	45.7	18.6	16.4	22.7	9.1

### 3.1.2. Variation with water depth

Mean DA concentrations in whole scallop and mean water depth for each of the four DA concentration groups 0–20, 20–50, 50–80 and >80 µg g<sup>−1</sup> are reported in Table 3. Using the pooled data a significant relationship was exhibited between water depth and mean DA concentrations in whole scallop ( $F_{1,2} = 44.93$ ,  $p < 0.001$ ).

Mean water depth over the 69 sampling stations ranged from 43 to 75 m. DA concentration in whole scallop exhibited a significant negative correlation with water depth ( $F_{1,67} = 94.26$ ,  $p = 0.000$ ,  $r = -0.765$ ) (Fig. 3). Similar negative correlations between water depth and DA concentration in hepatopancreas ( $F_{1,67} = 71.98$ ,  $p = 0.000$ ,  $r = -0.72$ ), adductor muscle ( $F_{1,67} = 47.96$ ,  $p = 0.000$ ,  $r = -0.65$ ), and remainder ( $F_{1,67} = 66.32$ ,  $p = 0.000$ ,  $r = -0.71$ ) were exhibited. There was low correlation between DA concentration in gonad and water depth ( $F_{1,67} = 5.92$ ,  $p = 0.018$ ,  $r = -0.29$ ). There was no correlation between DA concentration in gonad and gonad weight ( $F_{1,67} = 0.898$ ,  $p = 0.347$ ,  $r = 0.12$ ).

In addition to categorising the sampling stations into four DA concentration groups (Table 3), sampling stations were also categorised into four spatial groups using as determinants the lines at north/south 51.8° longitude and east/west at 7.0° latitude. These groups correspond to four visual clusters of the 69 stations and substantial agreement with this grouping was predicted by a cluster analysis procedure (Euclidean distance metric, average linkage). This spatial grouping also provided an inshore and offshore grouping of the stations. The spread of water depths and DA concentration in whole scallop for the four sample location groups is provided in Fig. 4A and B, respectively. Both offshore groups had a narrow range of water depths and DA concentrations. The inshore west group had a wide range

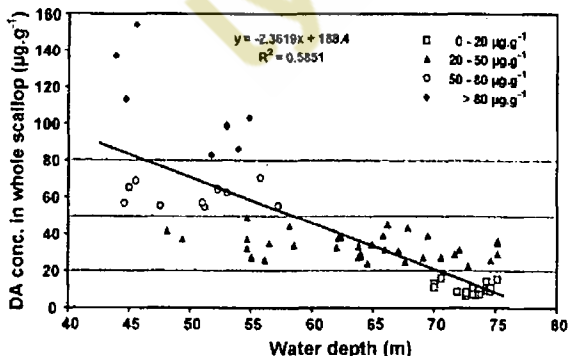


Fig. 3. Variation in DA concentration in whole scallop *P. maximus* off the southeast coast of Ireland with water depth.

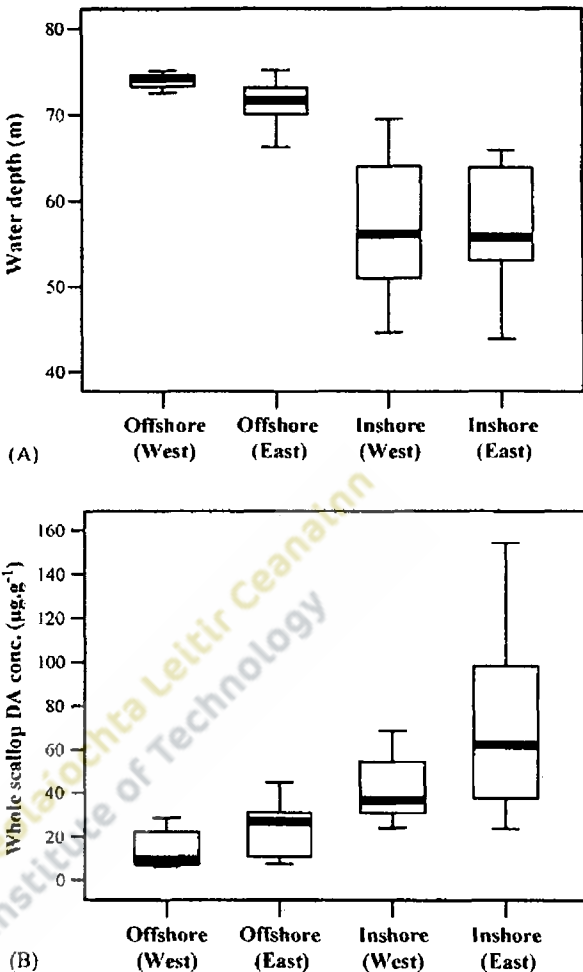


Fig. 4. Box plots showing the spread of water depth (A) and DA concentration in whole scallop (B) in the four sampling groups based on geographical location.

of water depths and a wide range of DA concentrations. By contrast the inshore west group had a wide range of water depths but a narrow range of DA concentrations.

### 3.1.3. Variation with scallop length, weight and age

Scallops from the four concentration groups were of commercial size and had similar mean physical characteristics, viz, shell length, shell height and age. Mean DA concentration in whole scallop in the four groups showed a significant relationship with mean total tissue weight ( $F_{1,2} = 48.95$ ,  $p = 0.02$ ). Increased total tissue weight in the four concentration groups was attributed to significant increases in gonad weight with a smaller contribution from increased adductor muscle weight (Table 3).

The relationships between mean DA concentration in whole scallop at the 69 sampling stations and shell length, total tissue weight and age was investigated. Shell lengths



ranged from 100 to 119 mm (mean 109.3 ± 4.3 mm), total tissue weights ranged from 21.5 to 75.1 g (mean 44.5 ± 8.8 g) and age ranged from 3 to 7 years (mean 4.9 ± 0.7 years). DA concentration in whole scallop exhibited no correlation with shell length ( $F_{1,67} = 0.238$ ,  $p = 0.627$ ,  $r = 0.06$ ) or age ( $F_{1,67} = 1.143$ ,  $p = 0.289$ ,  $r = 0.13$ ), however there was some correlation with total tissue weight ( $F_{1,67} = 32.45$ ,  $p = 0.000$ ,  $r = 0.57$ ).

3.2. Variation in DA concentration with scallop size

Mean shell length, mean DA concentration in hepatopancreas based on individual analysis and mean DA concentration in gonad, adductor muscle and tissue remainder based on composite samples for each of four scallop size groups from the five stations shown in Fig. 2 are provided in Table 4.

Comparisons of mean DA concentration in hepatopancreas of small, medium, large and very large scallops within each of the five sampling stations are

illustrated in Fig. 5. Coefficients of variation for DA concentration in hepatopancreas ranged from 24% to 65% (Table 4). Significant differences between DA concentration in hepatopancreas between the four size groups at each of the stations 5 ( $F_{1,2} = 3.915$ ,  $p = 0.015$ ), 36 ( $F_{1,1} = 4.992$ ,  $p = 0.013$ ), 44 ( $F_{1,2} = 12.940$ ,  $p = 0.000$ ) and 70 ( $F_{1,2} = 4.588$ ,  $p = 0.007$ ) were exhibited, however station 94 did not exhibit significant statistical differences between the size groups ( $F_{1,2} = 1.375$ ,  $p = 0.266$ ).

The relationship between individual DA concentration in hepatopancreas and individual shell length from each of the five stations was investigated without categorising into size groups. At stations 5 ( $F_{1,46} = 13.348$ ,  $p = 0.001$ ,  $r = 0.47$ ), 36 ( $F_{1,35} = 7.598$ ,  $p = 0.009$ ,  $r = 0.43$ ) and 44 ( $F_{1,46} = 18.761$ ,  $p = 0.000$ ,  $r = 0.54$ ) significant correlations were exhibited. At stations 70 ( $F_{1,42} = 0.086$ ,  $p = 0.771$ ,  $r = 0.05$ ) and 94 ( $F_{1,38} = 0.547$ ,  $p = 0.464$ ,  $r = 0.12$ ) the correlations were not significantly different from zero.

Table 4  
Variation in DA concentration in tissues of different sizes of scallops *Pecten maximus* from five sampling stations off the southeast coast of Ireland

			Individual samples		Composite samples		
			Hepatopancreas		Gonad	Adductor	Remainder
	<i>n</i>	Mean shell length ± S.D. (mm)	Mean DA conc. ± S.D. (µg g <sup>-1</sup> )	%CV	Mean DA conc. (µg g <sup>-1</sup> )	Mean DA conc. (µg g <sup>-1</sup> )	Mean DA conc. (µg g <sup>-1</sup> )
Station 5							
Small	12	79.2 ± 2.1	265 ± 146	55	21.2	13.5	36.1
Medium	12	87.9 ± 2.5	412 ± 267	65	31.2	11.8	78.1
Large	12	110.0 ± 4.5	480 ± 135	28	15.7	8.7	22.3
Very large	12	123.2 ± 3.8	532 ± 231	43	18.6	6.0	13.0
Station 36							
Small	12	79.2 ± 2.6	231 ± 119	51	10.6	17.6	49.0
Medium	12	93.3 ± 4.9	308 ± 97	32	17.9	8.1	44.5
Large	12	105.8 ± 4.8	363 ± <sup>a</sup>		22.2	8.5	20.3
Very large	12	121.5 ± 3.0	389 ± 145	37	23.1	13.0	27.5
Station 44							
Small	12	82.6 ± 3.2	109 ± 39	36	10.2	8.1	30.7
Medium	12	94.3 ± 4.5	314 ± 103	33	13.8	14.1	15.1
Large	12	111.2 ± 3.2	287 ± 88	31	19.7	4.2	10.5
Very large	12	119.7 ± 1.9	340 ± 143	42	20.3	7.7	12.9
Station 70							
Small	12	74.7 ± 6.9	44 ± 22	50	12.0	4.2	4.9
Medium	12	97.8 ± 1.5	28 ± 8	27	11.4	2.2	5.7
Large	12	107.4 ± 4.8	53 ± 21	39	5.3	0.8	2.7
Very large	12	121.2 ± 1.8	37 ± 14	37	9.1	2.0	9.9
Station 94							
Small	12	76.6 ± 4.2	203 ± 49	24	20.8	14.3	18.4
Medium	12	94.1 ± 2.5	270 ± 101	37	24.1	8.9	21.1
Large	12	107.1 ± 3.2	270 ± 93	34	30.2	5.5	14.6
Very large	12	123.0 ± 3.6	236 ± 94	40	29.3	4.9	10.0

<sup>a</sup> Not available due to a sample processing error.



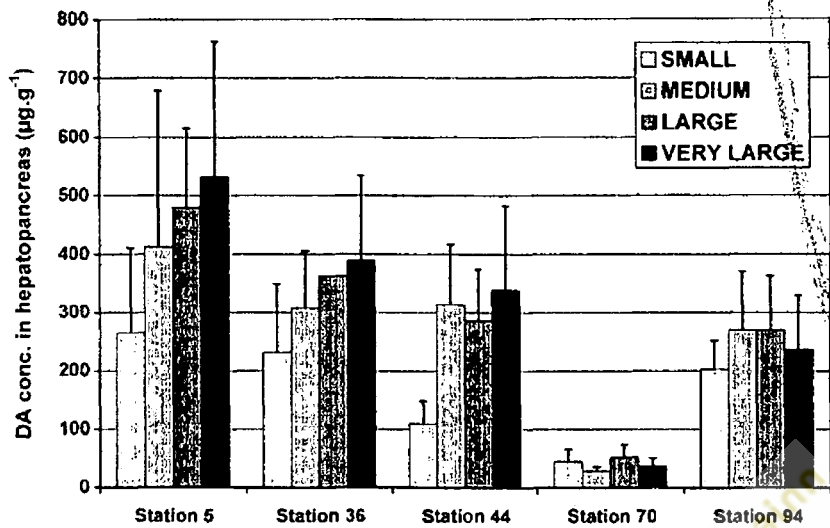


Fig. 5. Variation in DA concentration in hepatopancreas of four different sizes of king scallop from five sampling stations off the southeast coast of Ireland.

Based on these results, stations 5, 36 and 44 were considered as one inshore area and stations 70 and 94 as one offshore area (Fig. 2). Comparisons of mean DA concentration in hepatopancreas of small, medium, large and very large scallops within the inshore and offshore areas are illustrated in Fig. 6. The data pooled by size groups in the inshore and offshore areas suggested an increased DA concentration with size in the inshore area but no such relationship in the offshore area.

Consideration of individual data from inshore and offshore areas showed that significant, but weak correlation existed between DA concentration in hepatopancreas and shell length in the inshore area ( $F_{1,131} = 27.712$ ,  $p = 0.000$ ,  $r = 0.42$ ) but not in the

offshore area ( $F_{1,82} = 0.108$ ,  $p = 0.743$ ,  $r = 0.04$ ). Comparison of mean DA concentration in hepatopancreas of scallops from the inshore area with mean DA concentration in hepatopancreas of similar-sized scallops from the offshore area showed a significant difference ( $p = 0.043$ ) for the small size group and highly significant differences for the medium, large and very large size groups ( $p \leq 0.001$ ).

DA concentrations in composite samples of gonad from each size group at each station showed wide variation and exceeded the regulatory limit ( $20 \mu\text{g g}^{-1}$ ) on numerous occasions (Table 4). At inshore stations 36 and 44, DA concentration in gonad followed the same trend as in hepatopancreas, i.e. DA concentration

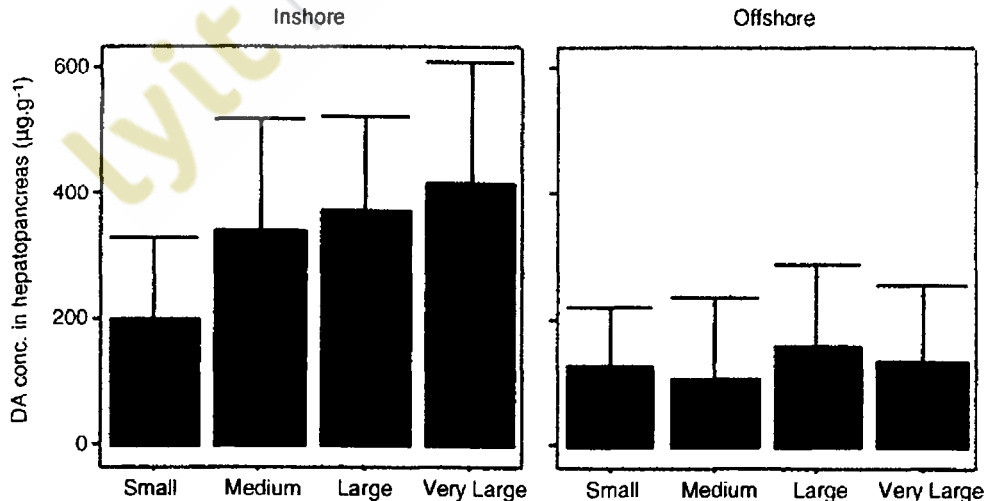


Fig. 6. Variation in DA concentration in hepatopancreas of four different size groups of scallop from inshore and offshore areas off the southeast coast of Ireland.

increased with scallop size. Concentrations of DA were highest in adductor muscle from small scallops at four of the five stations investigated but did not exceed  $20 \mu\text{g g}^{-1}$  in any sampling stations.

#### 4. Discussion

In this investigation DA, the toxin responsible for ASP, was measured at concentrations above the limit of detection in king scallop *P. maximus* from all sampling stations off the southeast coast of Ireland. Distribution of the toxin in scallop tissues was similar to that reported in other studies, highest concentrations occurring in hepatopancreas, followed by tissue remainder, gonad and adductor muscle (Arévalo et al., 1998; Campbell et al., 2001; Gallacher et al., 2001; Blanco et al., 2002).

Variability in DA concentration in king scallop tissues has been investigated at many different levels from sampling to analysis. These include inter-animal variation at the same sampling site (Arévalo et al., 1998; Campbell et al., 2001; Fryer et al., 2002), variation during tissue preparation (Hess et al., 2000; Campbell et al., 2003), between transportation and laboratory storage procedures (Vale and Sampayo, 2002; Smith et al., 2006) and between replicate samples during measurement (Hess et al., 2001, 2005). Variations associated with the analytical steps of the procedure were reported to be small by comparison with inter-animal variation (Fryer et al., 2002). In this study, comparison of the CVs from DA analysis of hepatopancreas, the tissue with the highest concentration and hence reduced probability of high CVs due to low DA concentration has been reported. Inter-animal variation in DA concentration of hepatopancreas from four samples, each of 12 large scallops (110–115 mm) exhibited CVs from 28% to 39% (Table 4). By comparison variation in DA concentration of the hepatopancreas from 69 sampling stations within one sampling area, each sample comprising a homogenate of 12 large scallops (100–110 mm) exhibited a much larger CV of 86.7%. This clearly demonstrated the significance of spatial variation within a single sampling area and the difficulties faced by toxin regulators in procuring samples representative of DA concentration.

Both spatial and inter-animal variability in DA concentration has been reported in three sampling areas on the west coast of Scotland in which three, four and five sampling stations, respectively, were investigated. DA concentrations in the gonad of king scallops exhibited CVs ranging from 53.9% to 78.7% in the three sampling areas, with inter-animal variation exceeding

this range (Campbell et al., 2001). A larger study of 10 sampling sites within one fishing box on the Scottish west coast also reported significant spatial and inter-animal variation in DA concentration in king scallop gonads (FSA, 2001).

In this study DA concentration in whole scallop tissue and individual tissues, with the exception of gonads, exhibited significant negative correlations with water depth. Similar relationships have been reported for both whole scallop and gonad tissues (FSA, 2001). The absence of a correlation between DA concentration in gonad and water depth in this study was attributed to difficulties in interpreting toxin concentration data based on this tissue since the measured DA concentration in gonad reflects a combination of the amount of DA in the intestinal loop and the mass of gonad tissue present at a particular stage of the reproductive cycle. It was initially suspected that the concentration of DA in gonad would decrease as the reproductive organ increased in size due to the dilution effect of gonad tissue on DA in the scallop intestine. Water depth has been shown to significantly affect gonad size attained during the reproductive cycle (Minchin and Ni Donnachada, 1995) and hence it was suspected that larger scallop gonads in shallower waters might exhibit reduced DA concentrations. While the concentration group data suggested that the converse occurred, i.e. as gonad weight increased so too did the concentration of DA in gonad, consideration of individual gonad data from all 69 sites revealed no correlation between gonad weight and DA concentration.

The negative correlation between DA concentration in whole scallop and individual tissues with water depth raised the question as to why such a relationship might exist. Shallower sampling stations exhibiting higher DA concentrations in this study were closer inshore, however they were also directly opposite Waterford Harbour and the possibility cannot be ignored that high DA concentrations may in part have resulted from geographical location rather than from water depth alone. Differences in DA concentration between inshore sites and offshore sites may also be influenced by the prevailing oceanographic conditions. Paralytic shellfish poison (PSP) toxin concentrations in sea scallop *Placopecten magellanicus* exhibited both seasonal and geographical variability between inshore (20 m depth) and offshore (180 m depth) samples, although high toxin concentrations were present throughout the year in the offshore zone compared to early summer peaks in toxicity in the inshore zone (Cembella et al., 1993). Variability in PSP toxin concentrations in geoduck clams *Panope abrupta* at different depths

may have resulted from variations in food availability caused by currents and winds (Curtis et al., 2000). In this study area off the southeast coast of Ireland the strongest currents occur in inshore waters particularly in the eastern part of the sampling area (Tully et al., 2002). These stronger currents may result in exposure to enhanced levels of phytoplankton, including toxic *Pseudo-nitzschia* cells, causing elevated DA concentrations, increased growth rates and larger gonads (Table 3) in scallops from inshore compared to offshore waters. The boxplots prepared for the spatial cluster groups at offshore east, offshore west and inshore east supported the relationship between DA concentration and water depth (Fig. 4). However inshore west sampling stations exhibited a narrow range in DA concentration but wide range in water depth suggesting that factor(s) other than water depth influenced DA concentration.

Several laboratory investigations have been undertaken which suggest that shellfish size may influence toxin concentration. Higher weight-specific filtration rates in smaller bivalves compared to larger bivalves (Jones et al., 1992) suggest that in the event of a toxic phytoplankton bloom, a period may exist when differential toxin concentrations might occur. Likewise depuration rates of DA from small mussels *M. edulis* (45–55 mm) were faster than in large mussels (60–70 mm) and even more rapid at higher temperatures (Novaczek et al., 1992). Field studies with king scallops have not reported any relationship between DA concentration and scallop size, although this may have been restricted by the limited size range of scallops under investigation; 80–100 mm (Arévalo et al., 1998) and 90–120 mm (Campbell et al., 2001). In this investigation, scallops ranging in size from 75 to 123 mm were analysed. Individual analysis of the scallop hepatopancreas rather than one of the other tissues was undertaken, since this organ with its higher DA concentrations relative to the other tissues should allow differences, if present, to be more easily confirmed. Mean DA concentrations for the four size groups at four of the five sampling stations (5, 36, 44 and 70) showed significant statistical differences. However the high level of inter-animal variability in DA concentration of the hepatopancreas in the size groups did not allow confirmation of a positive correlation with size, CVs ranging from 24% to 65% (Table 4).

Considering the three inshore stations, 5, 36 and 44 separately from the offshore stations, mean DA concentration in hepatopancreas increased with scallop shell length with the exception of one size group at one station (station 44, size group: large) (Fig. 5). When the

results were considered individually for each of the stations, a significant correlation existed between DA concentration in the hepatopancreas and scallop shell length at the three inshore stations (5, 36 and 44) but not at the offshore stations (70 and 94). These datasets emphasized the importance of considering all the results individually rather than only in size groups. On the basis of these similarities the three inshore stations were considered as one sampling area and the two offshore stations as a further sampling area. Data from the inshore area showed a significant correlation between DA concentration in the hepatopancreas and shell length in contrast to results reported by Arévalo et al. (1998) and Campbell et al. (2001).

To illustrate the effects of spatial variation in DA concentration on the status of this fishing area if Commission Decision 2002/226 were applied, results from each of the 69 samples were considered individually as the result required under this regulation (Table 2). Although any decision based on CD 2002/226 would necessitate two consecutive samples to be taken from an area before the fishing status is assigned, for the purposes of this illustration, this has been ignored. Sixteen of the samples had whole body DA concentrations less than  $20 \mu\text{g g}^{-1}$  and none of the edible parts individually exceeded this concentration indicating that the fishery would be open for harvesting. Four samples had a whole body DA concentration exceeding  $20 \mu\text{g g}^{-1}$  but less than  $250 \mu\text{g g}^{-1}$  and the adductor muscle and gonad contained less than  $4.6 \mu\text{g g}^{-1}$ , indicating that restricted harvesting with processing of the catch into a roe-on product (adductor and gonad) would be allowed. A further 24 samples had a whole body DA concentration exceeding  $20 \mu\text{g g}^{-1}$  but less than  $250 \mu\text{g g}^{-1}$  but only the adductor muscle contained less than  $4.6 \mu\text{g g}^{-1}$  indicating that restricted harvesting with processing of the catch into a roe-off product (adductor only) would be allowed. Twenty-five of the samples had a whole body DA concentration exceeding  $20 \mu\text{g g}^{-1}$  but less than  $250 \mu\text{g g}^{-1}$  but neither the adductor muscle or gonad contained less than  $4.6 \mu\text{g g}^{-1}$  indicating the fishery would be closed for harvesting. Clearly given the range of outcomes for the status of this scallop fishery if Commission Decision 2002/226 were to be applied, from open for harvesting, to restricted harvesting status with two levels of product processing, to a closed fishery, an improved understanding of the factors responsible for this variation are required to allow the collection of representative samples and better informed decisions as to when to open and close such a fishery.

In Washington State emergency closures of the fishery for razor clams, *Siliqua patula*, in 1991, 1998



and 1999 resulted from high variability in the DA concentration associated with sampling (CV 122%,  $n = 445$ ) (Wekell et al., 2002). Recommendations for improvements in the sampling strategy included increasing the sample size from 6 clams to at least 12, and if feasible to 24, to address the issue of high inter-animal variability. Implementation of a similar strategy to increase the number of scallops comprising a sample from the ten individuals or edible parts recommended in Commission Decision 2002/226 to a higher number would be likely to reduce the influence of inter-animal variability but would not address the issue of spatial variability highlighted in this study. To address the issue of spatial variability in the razor clam study it was suggested that sample collection might be focussed on sites associated with elevated DA concentrations to ensure the collection of clams with highest toxicity. Application of such an approach by countries using area management of scallop fisheries on the basis of these results would inevitably result in a higher incidence of closures, however from a risk management viewpoint, the likelihood of exposure to DA toxicity would be minimised. If the spatial variability in DA concentration exemplified in this study between offshore and inshore areas was consistent and typical of scallop fisheries, some consideration might be warranted by those countries applying Commission Decision 2002/226 towards reduction in the size of the sampling areas used for monitoring and management purposes, though such reduction would clearly depend on the economic significance of the scallop fishery under consideration. Although both sampling and analytical costs would be increased by the use of a larger number of smaller-sized sampling areas, such action would allow both improvements in risk management and a reduction in the incidence of unnecessary closures for the scallop industry. A similar recommendation that harvesting areas be treated individually on the basis of their toxin concentration and water depth was proposed to address variations in the concentration of PSP toxins in geoduck clams at different depths (Curtis et al., 2000).

In Ireland the issue of spatial variation in DA concentration in the wild scallop fishery, the high risk of false negatives and false positives and the effects that such outcomes could have on both consumer safety and the incidence of scallop fishery closures is addressed by using a landings-based approach rather than an area-based approach to scallop fishery management. Upon landing all catches from the wild scallop fishery, whether from the Mine Head and Saltees grounds in the southeast, the southern Irish Sea or other grounds, must be

dispatched to the processing factory, shucked, the hepatopancreas removed and the roe-on product frozen. A representative sample from each landing must be analysed for DA concentration and no product is allowed onto the marketplace before test results demonstrate that the DA concentration in both adductor and gonad is below  $20 \mu\text{g g}^{-1}$ . In summary confidence in the safety of Irish scallops from the wild fishery is maintained by testing a representative sample of frozen product from each landing and not allowing access to the fresh market. Given the pattern of spatial variation exhibited in this study performed between September/October 2003, seafood safety considerations suggest that the existing landings-based approach be maintained. Should a similar pattern in DA concentration be exhibited in a further study in this scallop fishery, which currently accounts for 90% of Irish scallop landings, consideration might be warranted in this special instance as to what management changes would be required to allow the sale of fresh scallop from this wild fishery.

## 5. Conclusions

This study highlights the difficulties faced by regulators using area-based biotoxin management schemes in procuring representative samples when DA concentration in whole scallop ranged from 6.5 to  $154.3 \mu\text{g g}^{-1}$  within an area less than that of a single ICES box. In this case study, toxin concentration was highest in inshore waters close to Waterford Harbour and lowest in deeper offshore waters. A significant negative correlation between toxin concentration and water depth was recorded. DA concentration in hepatopancreas of scallops from inshore waters exhibited a significant positive correlation with scallop size but no relationship in offshore waters. Further studies are required to investigate if the pattern in spatial variation in the southeast scallop fishery reported is fixed or transient.

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### Appendix 3. Paper 3

Comparison of domoic acid concentration in king scallops, *Pecten maximus* from seabed and suspended culture systems. J. Shellfish Res. 25 (1), 129-135.

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#### Author Contributions

Y.M.B., J.W.S. and P.H. designed the research programme.

Y.M.B. and A.L.H. prepared the scallop tissues.

Y.M.B. extracted the toxin and performed the research study.

J.G. provided technical support.

Y.M.B., J.W.S. and D.J.K. analyzed the data.

Y.M.B., P.H. and J.W.S. prepared the manuscript.



## COMPARISON OF DOMOIC ACID CONCENTRATION IN KING SCALLOPS, *PECTEN MAXIMUS* FROM SEABED AND SUSPENDED CULTURE SYSTEMS

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**ABSTRACT** Domoic acid (DA) the toxin responsible for amnesic shellfish poisoning (ASP) has proven problematic for king scallop *Pecten maximus* fisheries and aquaculture in Ireland. Toxin concentration in hepatopancreas of individual scallops and composite samples of gonad and adductor muscle of scallops suspended from a submerged longline, 2 m beneath the water surface and on a seabed site, 12–15 m beneath the suspended scallops were monitored from February 2003 to February 2004 at an aquaculture site in Clew Bay, Ireland. DA concentration in hepatopancreas of scallops from the seabed and longline peaked in April 2003, individual concentrations reaching 1037.1  $\mu\text{g}\cdot\text{g}^{-1}$  and 1212.6  $\mu\text{g}\cdot\text{g}^{-1}$  respectively. No statistically significant differences were exhibited between DA concentration in hepatopancreas of seabed and longline scallops except on 1 of the 10 sampling occasions. Slow depuration of DA toxin from hepatopancreas occurred from April 2003 to June 2003 and the concentration remained relatively stable from June 2003 to Feb 2004. Interpretation of DA concentrations in gonad were complicated by the lower concentrations recorded and the variable size of the gonad caused by the reproductive cycle over the 12-month study duration. DA concentrations in adductor muscle were below the limit of detection throughout the investigation. In summary DA concentration between scallops held in suspension or maintained on the seabed exhibited minor difference, and routine monitoring samples could be collected annually and held in suspended culture systems rather than using more expensive diver collection for sample procurement.

**KEY WORDS:** amnesic shellfish poisoning (ASP), domoic acid, *Pecten maximus*, scallop

### INTRODUCTION

Amnesic shellfish poisoning (ASP) was first recorded in Canada in 1987 when over 100 people became ill after consuming mussels *Mytilus edulis* contaminated with the DA toxin. Early symptoms included nausea, gastroenteritis and vomiting followed within 48 h by neurological symptoms such as confusion, lethargy, disorientation and memory loss that persisted indefinitely; three of those affected in this outbreak died (Quilliam & Wright 1989, Todd 1993). The toxin, a small tricarboxylic amino acid belonging to the kainoid class of compounds, has been reported in 10 species of diatoms of the genus *Pseudo-nitzschia* (Bates 2000). Filter feeding bivalves such as scallops and mussels consuming these toxic phytoplankton species can accumulate the toxin to high concentrations (Zaman et al. 1997). Human consumption of shellfish contaminated with DA results in ASP.

Detection of DA in cultured mussels from Galicia, Spain in 1994 (Arévalo et al. 1997) and a range of Portuguese shellfish in 1995 (Vale & Sampayo 2001) led to the introduction of amendment 61/97 of EU Directive 91/492/EEC, which established the maximum allowable concentration of DA in whole shellfish or edible parts as 20  $\mu\text{g}\cdot\text{g}^{-1}$ . Extensive shellfishery closures have since been recorded in many European countries, perhaps the most significant being those applied to the king scallop fishery off the west coast of Scotland from 1999 onwards, which resulted in considerable financial hardship for scallop fishermen (Gallacher et al. 2001, Smith et al. 2005). Prolonged closures of scallop fisheries in Europe because of elevated DA concentration persisting in some instances for months or years (Arévalo et al. 1998, Fernandez et al. 2000), slow rates of depuration in this species, localization of the majority of toxin in the hepatopancreas and high inter-animal variation between scallops led to the introduction of EU Commission Decision 2002/226/EC. This allowed harvesting of scallops with a whole body DA concentration exceeding 20  $\mu\text{g}\cdot\text{g}^{-1}$  if the parts to

be marketed, principally the adductor muscle and gonad, contained less than 4.6  $\mu\text{g}\cdot\text{g}^{-1}$ . Scallop fishing from grounds that otherwise would have remained closed has been allowed, but this EU decision has necessitated that almost all the catch be processed in approved plants prior to sale. Restrictions on the sale of fresh scallops (in-shell), which have traditionally commanded a premium price compared with processed product, has resulted in a 2-tier market for fishermen, a high value one for fresh product, which can rarely be supplied and a lower value one for processed product.

In Ireland high DA concentrations in scallop recorded in late 1999 and early 2000 resulted in the closure of all scallop production/harvesting over the millennium period. Over the last 5 years, as in much of Europe, this toxin has proven particularly problematic in king scallop *Pecten maximus* fisheries. Recent DA concentrations of 618.2  $\mu\text{g}\cdot\text{g}^{-1}$  reported in April 2005 in mussels *Mytilus edulis* from the west coast of Ireland represented the first time the toxin had been detected above 50  $\mu\text{g}\cdot\text{g}^{-1}$  in this species. Interest in DA concentrations in scallop suspended in the water column compared with scallops on the seabed has been expressed from a number of perspectives. From an aquaculture perspective, scallops can be farmed either by sowing juveniles into seabed areas and harvesting at a later date or by more intensive culture in lantern nets suspended from submerged longlines. Both methods have been attempted in Ireland and in some bays both techniques have been used. Current legislation on the monitoring of DA refers to the number of shellfish to be used as a sample, the parts to be analyzed, the analytical technique to be used and the maximum concentrations. If differences in toxin concentration occurred between scallops produced by the two methods, then the possibility exists that scallops produced by one technique might be sold into a different market sector than the other, for example suspended scallops might have to be processed, whereas sown scallops from the seabed could be sold into the fresh market or *vice versa*. Given the higher value of fresh in-shell product, it is therefore conceivable that the production technique to be used in an area and the

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economics of scallop farming might be influenced by the likely toxin concentration in finished product. Such a possibility also raised the question that perhaps suspending scallops in shallower, phytoplankton-rich waters might increase rates of toxin depuration. Although bivalves that are actively feeding on nontoxic algae are likely to exhibit faster depuration (Bricelj & Shumway 1998), when mussels *Mytilus galloprovincialis*, contaminated with PSP toxin, okadaic acid, were transferred to a toxin-free zone the rate of depuration did not decrease significantly (Blanco et al. 1999). Nevertheless, the possibility that maintaining scallops in suspended culture nets until DA concentrations decreased to levels that allowed their sale into the more lucrative fresh market is worthy of further investigation for those faced with regular shellfish closures. The converse, whereby scallops suspended in waters containing a higher concentration of toxic species might accumulate more toxin than scallops on the seabed, should also be considered as a possible outcome of suspending scallops in the water column. Diver collection of farmed scallop has been proposed as a harvesting technique from intensive seabed plots. Though efficient as a means of scallop collection, such a technique is a slow method of harvesting, necessitating the holding of scallops for a period while a consignment is gathered. Concern has been expressed that during this holding period, scallops may accumulate toxins to levels such that their sale into the more valuable fresh market was no longer an option.

From a regulatory perspective, interest in DA concentrations in suspended versus seabed scallops centered on sample collection. In some inshore aquaculture sites in Ireland, divers are used for the collection of scallops for routine toxin monitoring. Recently introduced safety regulations for professional divers have resulted in an escalation of the costs associated with sample procurement using such methods. In circumstances where no differences existed between seabed and suspended scallops, sample provision for routine monitoring could be performed much more economically using scallops maintained for the purpose in suspended culture nets.

In addition to DA production from a range of *Pseudo-nitzschia* species, a benthic diatom, *Nitzschia navis viridica* has recently been reported as a further source of the toxin (Kotaki et al. 2004). The distribution of this species is at present largely unknown. If in a comparative study of DA concentration in seabed and suspended scallops, higher toxin concentrations were recorded in seabed scallops, DA production by benthic species might be suspected as a possible contributory factor.

Given the significance of results regarding DA toxin concentration in seabed and suspended scallops to fishermen, aquaculture producers, regulators and scientists; this comparative study was undertaken over a 12-month period at an aquaculture site on the west coast of Ireland.

## MATERIALS AND METHODS

King scallop *Pecten maximus* were collected by diving from a seabed aquaculture site in Clew Bay, County Mayo off the west coast of Ireland and subdivided into 2 batches—a suspended batch, which was held in lantern nets 2 m below the water surface from a submerged longline and a seabed batch, which was returned to the seabed below the longline at a depth of approximately 12 m to 15 m (Fig. 1). Samples from both batches were provided for DA analysis at approximately monthly intervals from February 2003 to February 2004. Each sample comprised 12 individuals from each batch of mean shell length between 100 mm to 115 mm. On three

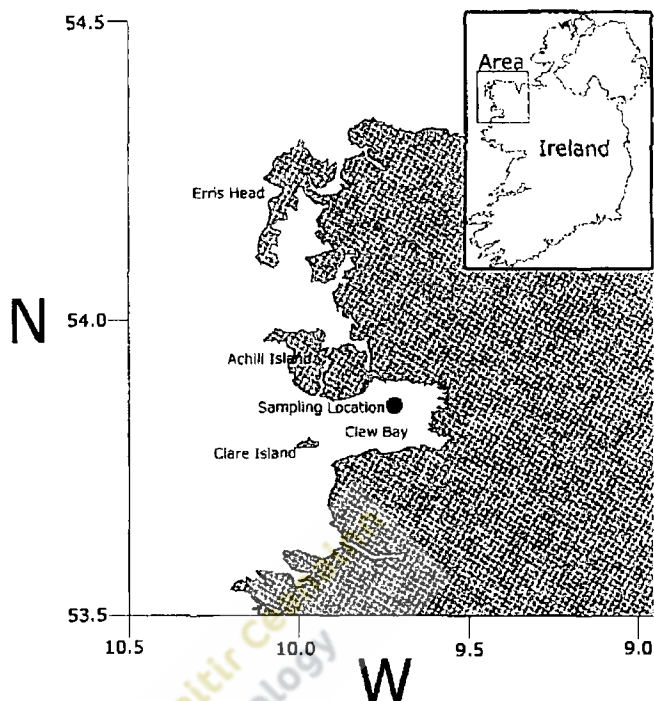


Figure 1. Sampling location in Clew Bay, County Mayo, Ireland.

occasions one or two shells in the longline sample were empty on arrival at the laboratory.

After shell measurement, scallops were dissected and the weight of total soft tissue and individual weight of hepatopancreas, gonad and adductor muscle recorded. The hepatopancreas of each scallop was analyzed individually to provide data on variability in DA concentration within each sample batch on each date. Composite samples of gonad and composite samples of adductor muscle from each sample group on each date were prepared for analysis, because previous studies suggested that DA concentration in these tissues would be much lower than in hepatopancreas; and hence differences, if present, between seabed and longline scallops would be more difficult to confirm because of the lower concentrations. Subsamples of composite tissues were analyzed in triplicate for DA toxin concentration.

DA was extracted from scallop tissue samples using procedures based on Quilliam et al. (1995) with several modifications. Extraction was performed from approximately 4.0 g of tissue homogenate with 16 mL of 50:50 extraction solvent (methanol:water) in a blender (Ultra Turrax T25, IKA-Works.) for 4 min at high speed. DA was extracted from each hepatopancreas individually. Where hepatopancreas weight exceeded 4.0 g, the organ was homogenized and a 4.0 g subsample used for extraction. After extraction, homogenates were centrifuged at 3,800 rpm for 30 min. A sample of supernatant was filtered using a methanol-compatible 0.45- $\mu$ m syringe filter and the combined concentration of DA and *epi*-DA in the filtered extract determined using a Shimadzu HPLC/UV and following equipment upgrade a Shimadzu HPLC/DAD (Mason Technology, Dublin). In those instances where the combined DA concentration exceeded that of the highest standard, the filtered extract was diluted with extraction solvent and the measurement of DA and *epi*-DA repeated. All solvents used were HPLC grade obtained from Lennox Laboratory Suppliers, Dublin. Mobile phase consisted of 10% acetonitrile and 1% TFA (trifluoroacetic acid) prepared with deionized water. HPLC flow rate was 0.5

mL.min<sup>-1</sup> and injection volume 20 µL. Calibration was performed externally using six DA calibration standards between 0.2 µg.mL<sup>-1</sup> to 10 µg.mL<sup>-1</sup> prepared from certified reference standard obtained from the NRC, Canada. Calibration curves were prepared for each sample batch and were always linear ( $R^2 > 0.999$ ).

Statistical analysis of all data was performed using SPSS, Version 12.

## RESULTS

Scallops used in this comparative study of DA toxin concentration were of a similar size (Table 1). Based on individual scallop measurements, the mean shell length and shell height for seabed scallops was  $108.8 \pm 3.9$  mm by  $96.4 \pm 3.9$  mm compared with  $111.6 \pm 5.2$  mm by  $99.4 \pm 4.6$  mm for longline scallops.

Mean DA concentration in the hepatopancreas of individual seabed scallops was  $326.4 \pm 169.9$  µg.g<sup>-1</sup> compared with  $313.9 \pm 214.6$  µg.g<sup>-1</sup> for longline scallops over the 12-month study at this location.

Mean DA concentrations in hepatopancreas and gonad of seabed and longline scallops on the 10 sampling occasions are provided in Table 1. DA toxin concentration in hepatopancreas of seabed and longline scallops exhibited similar trends over the 12-month duration (Fig. 2). The highest mean concentrations of DA in hepatopancreas were recorded on the second sampling occasion (April 3, 2003), individual DA concentrations reaching 1037.1 µg.g<sup>-1</sup> in seabed scallops and 1212.6 µg.g<sup>-1</sup> in longline scallops respectively. Comparison of the mean DA concentrations in hepatopancreas of seabed and longline scallops using a *t*-test demonstrated no statistically significant difference for 9 of the 10 samples. Only the sample from November 12, 2003 showed a significant difference ( $n = 23$ ,  $t = 3.263$ , sig. = 0.004) in the mean DA concentration between seabed scallops ( $298.3 \pm 89.4$  µg.g<sup>-1</sup>) and longline scallops ( $172.4 \pm 95.7$  µg.g<sup>-1</sup>).

DA concentrations in composite samples of gonad tissue never exceeded the regulatory limit of 20 µg.g<sup>-1</sup> and were considerably lower than concentrations recorded in hepatopancreas. Overall mean DA concentrations based on triplicate analysis of composite gonad samples were  $5.0 \pm 2.1$  µg.g<sup>-1</sup> in seabed scallops compared with  $3.8 \pm 1.9$  µg.g<sup>-1</sup> in longline scallops. DA toxin concentration

in gonads of seabed and longline scallops exhibited considerable fluctuations and dissimilar trends over the 12-mo study duration (Fig. 2). Because DA toxin concentration of gonads is influenced by both the mass of DA in the intestinal loop within the gonad and the mass of gonad tissue itself, the reproductive condition of scallops from both sample batches was determined in an attempt to understand the fluctuations and trends over the 12-mo study (Fig. 3). Relative gonad height (RGH) in both sample groups showed a similar trend with peak RGH at the end of May. Seabed scallops exhibited higher RGH than longline scallops, although differences between sample groups were not significant ( $n = 236$ ,  $t = 1.448$ , sig. = 0.149). The decline in RGH suggested a single prolonged spawning at this study location, although the use of monthly sampling for determination of the number of spawnings is not recommended (Slater 2005). Because of fluctuations in DA concentration within and between sample groups and varying reproductive condition over the 12-month study, the mass of DA in gonad was determined to examine if it better represented the changes in DA levels in gonad (Fig. 4). For comparative purposes the mass of DA in hepatopancreas was also determined (Fig. 4).

DA concentrations in composite samples of adductor muscle were below the limit of detection (LOD = 0.1 µg.g<sup>-1</sup>) in all samples over the 12-month study duration.

## DISCUSSION

DA concentrations in hepatopancreas were approximately 1 to 2 orders of magnitude greater than in gonad, hence if differences occurred between toxin concentration in longline and seabed scallops; such differences should be more easily recorded in those tissues having the higher DA concentration. Individual analysis of each hepatopancreas showed high inter-animal variability in DA concentration within each sample. Coefficients of variation (CV) of DA concentration in hepatopancreas of longline scallops ranged from 10.7% to 55.5% whereas that of seabed scallops ranged from 17.8% to 49.5%. The mean CV of DA concentration in hepatopancreas of longline and seabed scallops was 42.3% and 31.0% respectively. Similar high levels of inter-animal variability in DA concentration in king scallops have been reported (Campbell et al. 2001, Blanco et al. 2002, Bogan et al. 2006). Over the 12-month duration of the study similar DA concentrations in hepatopancreas

TABLE 1.  
Concentration of DA in hepatopancreas and gonad of seabed and longline scallops from Feb 03 to Feb 04.

	n	Seabed			n	Longline		
		Shell Length (mm)	Conc. of DA in HP (µg.g <sup>-1</sup> )	Conc. of DA in gonad (µg.g <sup>-1</sup> )		Shell Length (mm)	Conc. of DA in HP (µg.g <sup>-1</sup> )	Conc. of DA in gonad (µg.g <sup>-1</sup> )
26-Feb-03	12	112.91 ± 2.71	272.58 ± 59.77	2.00 ± 0.24	12	111.58 ± 4.19	283.67 ± 62.69	2.25 ± 0.03
03-Apr-03	12	108.54 ± 4.08	697.96 ± 156.55	5.35 ± 0.64	10	112.50 ± 2.46	837.70 ± 250.84	7.10 ± 0.23
23-May-03	12	109.17 ± 3.69	457.68 ± 134.61	4.07 ± 0.46	10	107.80 ± 2.90	452.53 ± 202.18	7.12 ± 0.30
03-Jul-03	12	107.67 ± 2.57	216.50 ± 107.22	2.99 ± 0.43	11	108.70 ± 5.31	296.05 ± 114.60	2.24 ± 0.24
30-Jul-03	12	108.08 ± 3.34	332.70 ± 140.64	6.07 ± 1.21	12	113.83 ± 5.29	265.90 ± 56.09	1.96 ± 0.98
03-Sep-03	12	109.91 ± 2.84	304.98 ± 69.39	6.72 ± 0.21	12	109.75 ± 4.67	262.16 ± 111.62	2.58 ± 0.06
25-Sep-03	12	111.91 ± 2.42	223.68 ± 65.37	8.45 ± 1.01	12	113.42 ± 5.87	210.45 ± 99.96	3.54 ± 0.26
12-Nov-03	12	110.08 ± 1.62	298.33 ± 89.42	3.55 ± 1.12	12	114.64 ± 6.83	172.42 ± 95.67	3.09 ± 0.15
04-Dec-03	12	104.50 ± 2.97	238.98 ± 109.41	6.56 ± 2.65	12	111.08 ± 5.71	208.72 ± 46.59	4.36 ± 1.12
11-Feb-04	12	104.36 ± 2.98	245.24 ± 43.75	4.02 ± 0.66	12	112.92 ± 4.99	244.55 ± 103.88	3.76 ± 0.79

Values represent the mean ± standard deviation.



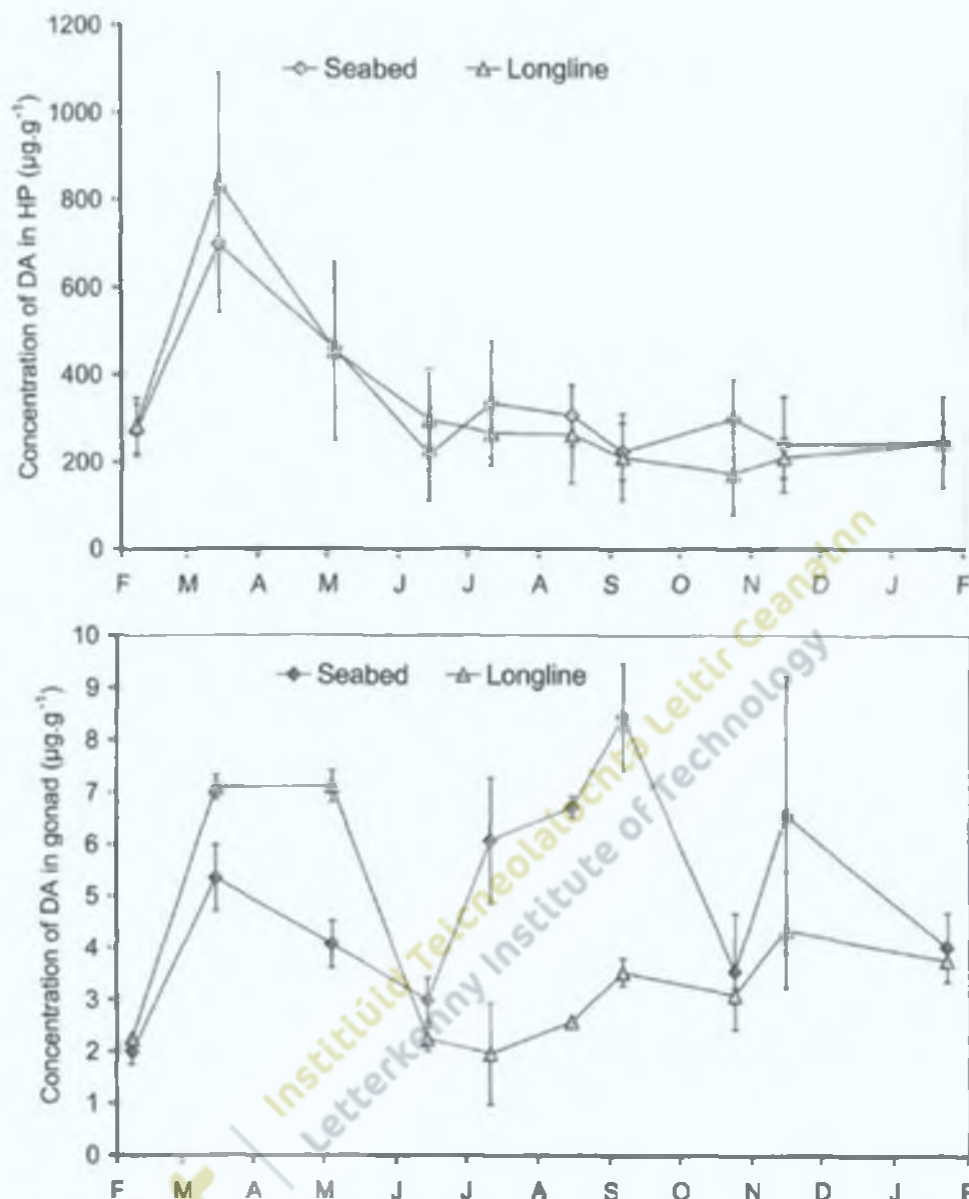


Figure 2. Concentration of DA in hepatopancreas (top) and gonad (bottom) of seabed and longline scallops from Feb 03 to Feb 04. (Each point represents Mean  $\pm$  St. Dev).

of longline and seabed scallops were reported and a statistical difference between the two sample hatches occurred on only one occasion. Similarly no significant differences in PSP toxin concentration were reported in a comparative study between sea scallops suspended 2 m beneath the water surface and those on the seabed at 11 m depth (Haya et al. 2003).

Over the 12-month duration of the study both seabed and longline scallops demonstrated rapid accumulation of DA in hepatopancreas in March 2003, slow depuration during April 2003 to June 2003 followed by relative stability from June 2003 to February 2004. The fact that there was no statistical difference in DA content of hepatopancreas between longline and seabed scallops was somewhat unexpected. Reports of toxic *Pseudo-nitzschia* cells descending after a bloom from nutrient-poor surface waters into higher-nutrient mixed layers suggested that differential profiles in toxicity might have been anticipated between suspended and seabed scallops (Dortch et al. 1997, Trainer et al. 1998, 2000). A time

delay in PSP toxin accumulation in suspended and seabed scallops was suggested as toxic cells in surface waters descended towards the seabed as the toxic bloom subsided (Bricelj & Shumway 1998). In this study no temporal difference in peak DA concentration in hepatopancreas between longline and seabed scallops was recorded although this may have been caused by the long sampling intervals between February 26, 2003 to April 3, 2003 and April 3, 2003 to May 23, 2003.

Blooms of toxic *Pseudo-nitzschia* cells are often regarded as being widely dispersed throughout the water column, however they have also been reported as thin layers in the water column and following sinking as near-bottom thin layers, which may extend over large areas. Cell densities have been reported within these thin layers exceeding  $10^6$  toxic cells.l<sup>-1</sup> (Rines et al. 2002). The absence of a significant difference in DA concentration in hepatopancreas between longline scallops suspended 2 m beneath the water surface and seabed scallops from 12 m to 15 m depth, in all

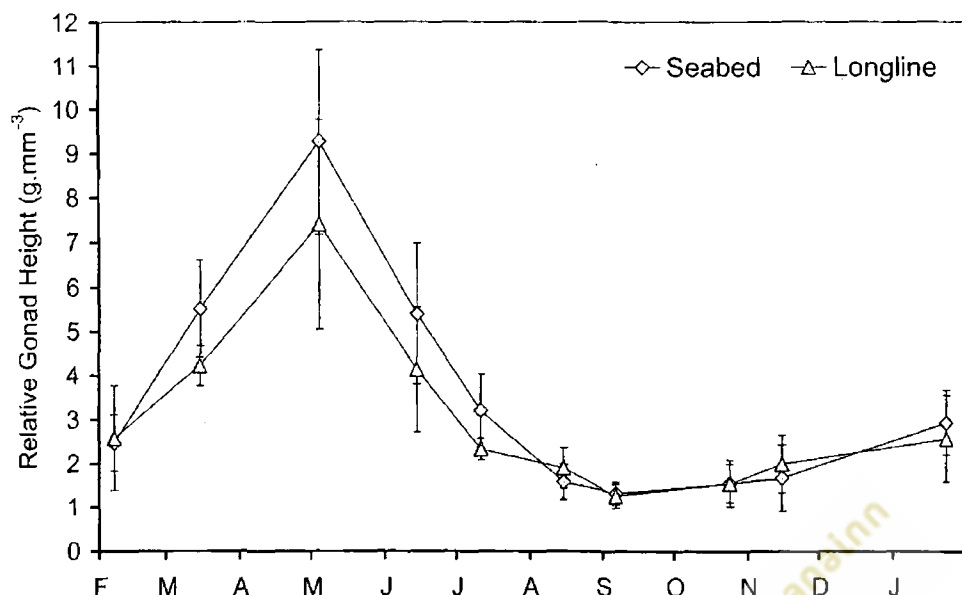


Figure 3. Relative gonad height of seabed and longline scallops from Feb 03 to Feb 04. (Each point represents Mean  $\pm$  St. Dev.).

but one of the samples, suggested that the source of DA toxicity was relatively evenly distributed throughout the water column in this location.

After the depuration phase during April 2003 to June 2003, concentrations of DA in the hepatopancreas over the remaining 8 month (June 2003 to February 2004) were relatively stable with minor fluctuations between  $170 \mu\text{g.g}^{-1}$  to  $330 \mu\text{g.g}^{-1}$  suggesting that no further depuration of toxin from hepatopancreas occurred, rates of uptake and depuration of DA were balanced or that the rate of depuration was very slow and not recorded over the 8-month period. Prolonged toxicity over an extended period rather than a continual decline in toxicity may have arisen from the continued development of small pulses of toxic *Pseudo-nitzschia* cells. Such pulses can result from *Pseudo-nitzschia* cells that had sunk after nutrient depletion being brought back into surface waters during upwelling events (Trainer et al. 2000). This replenishment of cells into surface waters has been proposed as a mechanism to explain why some blooms appear to persist for up to 3 mo in the field and might also explain why toxicity persisted throughout this study (Bates et al. 1989, Smith et al. 1990). Extended periods of toxicity attributed to very slow rates of depuration have also been reported in sea scallops, *Placopecten magellanicus* with PSP toxin over a 12-month period (Haya et al. 2003).

Many strains of domoic acid-producing diatoms have been reported, all belonging to the genus *Pseudo-nitzschia* except the benthic species *Amphora coffeaeformis* (Agardh) Kützinger (Bates 2000, Shimizu et al. 1989). Recently a benthic diatom, *Nitzschia navis-varingica*, collected from brackish water in Vietnamese shrimp ponds and identified as a new species has been demonstrated to be capable of DA production (Kotaki et al. 2000, 2004). Distribution of this benthic species is presently unknown although it has been reported in Japan and the Philippines and can be found in the water column (Lundholm & Moestrup 2000, Kotaki et al. 2004). The scallop toxicity results over a 12-month sampling period in this site provided no support for the hypothesis that DA production by benthic diatoms occurred.

DA concentrations in gonad were below the regulatory limit of  $20 \mu\text{g.g}^{-1}$  throughout the study and never exceeded  $10 \mu\text{g.g}^{-1}$  in

any composite sample of seabed or longline scallop gonad. Early results during the toxin accumulation phase in the hepatopancreas (March 2003) showed an expected increase in DA concentration in gonad. A corresponding decrease in DA concentration in gonad occurred from March 2003 to June 2003, although this was somewhat slower to commence in seabed scallops. During the period of relative stability in DA concentration in hepatopancreas (June 2003 to February 2004), DA concentrations in gonad fluctuated considerably and differences were exhibited between the two groups of scallops. To understand these fluctuations and between-group differences, the reproductive condition of the scallops using relative gonad height, a measure selected because it accounted for small differences in size between the scallop samples was examined. Because the DA is predominantly contained within the intestinal loop that passes through the gonad, it was expected that as the gonad increased in size DA concentration of gonad would decrease and vice versa. The results from the February 2003 to July 2003 period demonstrated the converse, as RGH increased and then declined after spawning, DA concentration in the gonad followed a similar trend. The results demonstrated that despite the variations in gonad size, DA concentration in the gonad was predominantly determined by DA concentration in the hepatopancreas, which exhibited a similar trend over the February 2003 to July 2003 period. The influence of gonad size on DA concentration in gonad was demonstrated by comparing toxin concentration between the scallop batches, higher RGH of seabed scallops being reflected in lower DA concentration whereas lower RGH of longline scallops was reflected in comparatively higher DA concentration. Fluctuations in DA concentration in gonad from Jul 03 to Feb 04 or variations between the two groups of scallop could not be explained by consideration of the RGH independently. In an attempt to explain the variations in DA concentration in gonad and gonad size, changes in the mass of DA in gonad were examined over the study duration. For comparative purposes changes in the mass of DA in hepatopancreas were also examined. Changes in the mass of DA in gonad over the 12-month period exhibited a similar trend to that for both the concentration and mass of DA in hepatopancreas. Because fluctuations in DA concentration of gonad from

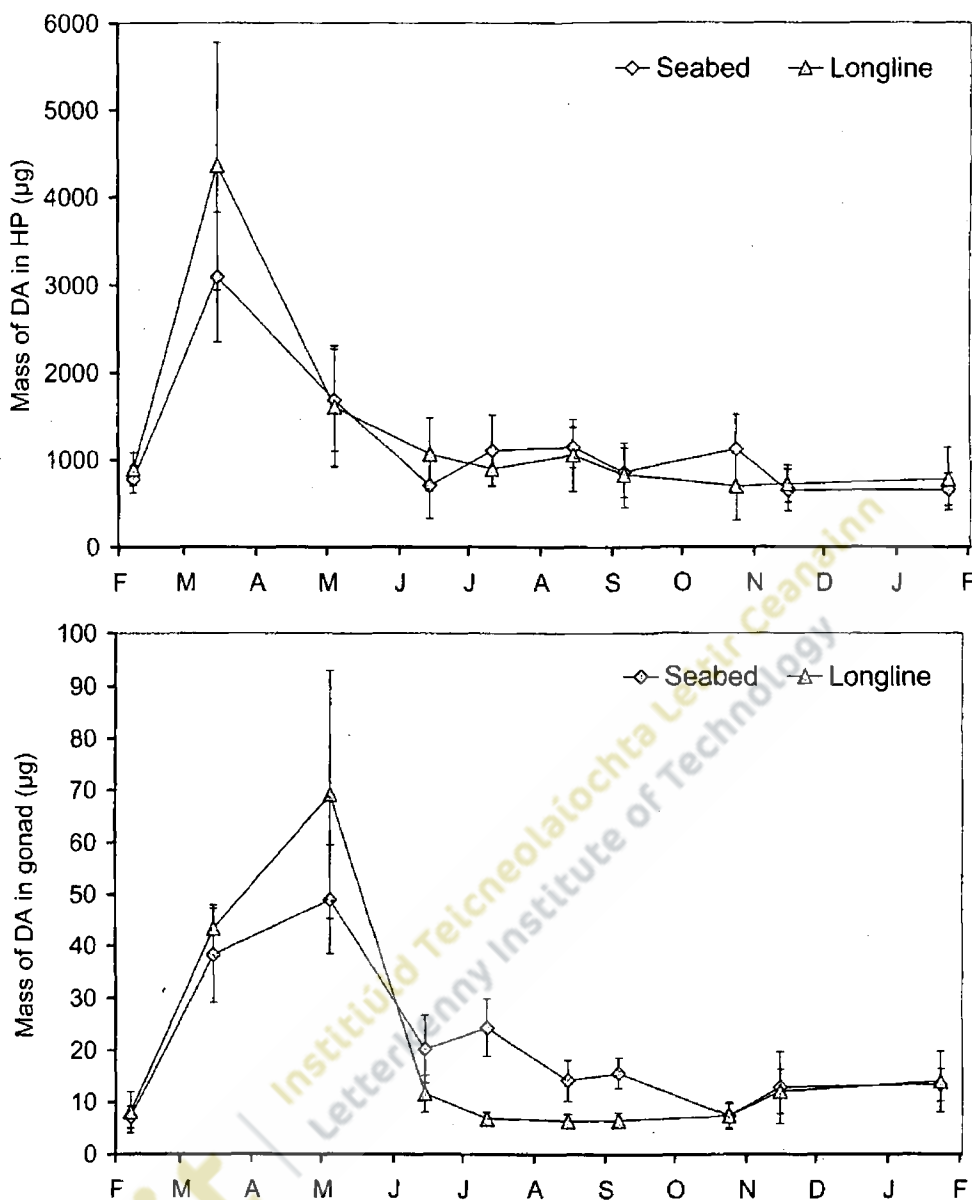


Figure 4. Mass of DA in hepatopancreas (top) and gonad (bottom) of seabed and longline scallops from Feb 03 to Feb 04. (Each point represents Mean  $\pm$  St. Dev.).

July 2003 to February 2004 could not be attributed to changes in gonad size, it was concluded that fluctuations during this period, and variations between the two scallop batches must have resulted from high inter-animal variability in DA concentration in gonad and experimental error. DA concentrations in gonad have exhibited high inter-animal variability in several other studies (Arévalo et al. 1998, Campbell et al. 2001). Similar difficulties to those encountered in this study in interpreting changes in DA concentration in gonad have been reported elsewhere (FSA 2001). Although regulatory monitoring of DA concentration in gonad for determining its suitability for entering the food chain is vital, the low concentrations compared with other tissues, variations in go-

gonad size during the reproductive cycle and difficulties encountered interpreting results, suggest that its value in understanding temporal changes is questionable.

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## **Appendix. Paper 4**

Variation in domoic acid concentration in king scallop (*Pecten maximus*) from fishing grounds around the Isle of Man. Harmful Algae.

Bogan, Y.M., D.J. Kennedy, A.L. Harkin, J. Gillespie, B. J. Vause, B.D. Beukers-Stewart, P. Hess. J.W. Slater. 2006.

### **Author contributions**

Y.M.B. and J.W.S. designed the research programme.

B.J.V. and B.D.B-S collected the scallops.

Y.M.B. and A.L.H. prepared the scallop tissues.

Y.M.B. extracted the toxin and performed the research study.

J.G. provided technical support.

Y.M.B., J.W.S. and D.J.K. analyzed the data.

Y.M.B., B.D.B-S., P.H. and J.W.S. prepared the manuscript.

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## Variation in domoic acid concentration in king scallop (*Pecten maximus*) from fishing grounds around the Isle of Man

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### Abstract

Domoic acid (DA), the toxin responsible for amnesic shellfish poisoning (ASP) can accumulate in king scallop *Pecten maximus* leading to extensive fishery closures. Approximately 59% of the total value of all fish and shellfish landed in the Isle of Man in 2004 comprised king scallop, hence the economy of the Manx marine sector is particularly susceptible to impacts from this biotoxin. Scallop from fishing grounds around the Isle of Man were sampled in October 2003, June 2004 and October 2004 to determine levels of inter-animal and spatial variability in DA concentration and factors that might influence toxin concentration such as scallop size and water depth. Mean DA concentrations in hepatopancreas ranged from 296.3  $\mu\text{g g}^{-1}$  to below the detection limit, in gonad from 27.8  $\mu\text{g g}^{-1}$  to below the limit of detection and in adductor muscle from 7.3  $\mu\text{g g}^{-1}$  to below the limit of detection. High levels of inter-animal variability of DA concentration in hepatopancreas were recorded; CVs ranging from 16.1% to 70.0%. DA concentrations above 20  $\mu\text{g g}^{-1}$  were recorded in gonads on all three sampling dates. Scallops from fishing grounds on the east of the Isle of Man were significantly less contaminated than those from the west and southwest. A significant positive correlation between DA concentration and shell length was recorded in some sites, but there was no relationship with water depth. The high inter-animal, spatial and seasonal variability in toxin concentration highlighted the importance of understanding field variability for the development of reliable sampling and management protocols.

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**Keywords:** Amnesic shellfish poisoning; Domoic acid; *Pecten maximus*; Spatial variability; Isle of Man

### 1. Introduction

A commercial fishery for king scallop *Pecten maximus* has existed around the Isle of Man since 1937 (Beukers-Stewart et al., 2003). The fishery constitutes a significant natural resource with harvesting by

boats from as far away as County Wexford in Ireland and Cornwall in England. Landings in the Isle of Man in 2004 amounted to 1106 tonnes valued at £1.7 million and accounted for approximately 59% of the total value of all fish and shellfish (Brand et al., 2005). Regulation of the fishery is achieved using a range of harvesting permits, a minimum landing size of 110 mm shell length, some 10 mm greater than the EU minimum size limit, an annual closed season extending from 1 June to 31 October, numerous restrictions applied to the fishing

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gear and more recently EU regulations concerned with biotoxins (Brand, 2000; Brand et al., 1991, 2005; Beukers-Stewart et al., 2003).

Production of domoic acid (DA), the biotoxin responsible for amnesic shellfish poisoning (ASP) has been reported in 10 species of diatoms within the genus *Pseudo-nitzschia* and more recently in a benthic *Nitzschia* species (Bates, 2000; Kotaki et al., 2000, 2004). Filter feeding bivalves such as scallops and mussels consuming these toxin-producing phytoplankton species can accumulate DA to high concentrations (Zaman et al., 1997). Human consumption of shellfish contaminated with DA can result in amnesic shellfish poisoning (ASP). This condition was first recorded in Canada in 1987 when three people died and over one hundred became ill after consuming blue mussels *Mytilus edulis* from Prince Edward Island contaminated with the toxin (Quilliam and Wright, 1989; Todd, 1993). In European waters, DA toxicity has impacted particularly on king scallop fisheries. Accumulation and prolonged retention of toxin by this species has resulted in extensive harvesting closures, perhaps the most significant being those applied in Scotland (Arévalo et al., 1998; Gallacher et al., 2001). To ensure the safety of shellfish for human consumption EU Amendment 97/61 to Directive 91/492/EEC established the maximum allowable concentration of DA in whole shellfish or edible parts as  $20 \mu\text{g g}^{-1}$ . Although not a European Member State, the Isle of Man exports shellfish to the United Kingdom and Europe and has adopted similar management protocols to those detailed in EU Amendment 97/61. During 2002 and 2003, DA concentrations in king scallops from Manx waters frequently exceeded the maximum allowable concentration (Shammon et al., 2004). Dredging has continued but shucking restrictions, similar to those in EU Commission Decision 2002/226 have been applied; allowing prepared but not whole shellfish to be placed on the market provided ASP toxin concentrations in the adductor muscle and gonad remain below threshold levels (Shammon et al., 2004).

High inter-animal and spatial variability in DA concentration has been widely reported in king scallops making management of fisheries affected by this toxin difficult (Arévalo et al., 1998; Campbell et al., 2001; FSA, 2001; Bogan et al., 2006a, 2006b). Quantification of the extent of this variability and an understanding of its causes are important criteria for the development of reliable sampling and management protocols. In the Isle of Man, routine monitoring of ASP toxicity in scallops since 2002 has demonstrated considerable inter-annual and spatial variation; fishing grounds to the west and southwest exhibiting higher toxin concentrations

(Shammon et al., 2004). The objectives of this study were to measure DA concentrations in king scallop from around the Isle of Man and to assess the variability both within and between different fishing grounds.

## 2. Materials and methods

### 2.1. Sample collection

For stock assessment purposes, scallop-fishing grounds around the Isle of Man are surveyed just before the start of each scallop fishing season (31 October) and just after the end (1 June). Three or four replicate tows (each 2 nautical miles long) were performed on each ground using spring-loaded Newhaven type dredges. Samples for DA analysis were collected from six fishing grounds on the first sampling occasion (mid-October 2003) and from eight fishing grounds on subsequent sampling occasions (mid-June 2004 and end-October 2004). As far as possible, tows covered the same area of seabed on each survey. Samples comprising 12 scallops from each of the fishing grounds: Targets, Peel, Bradda Inshore, Bradda Offshore, Chickens, 15 Miles South-Port St. Mary, East Douglas and Laxey, except for October 2003 when samples from Chickens and 15 Miles South-Port St. Mary were not available, were frozen at  $-20^\circ\text{C}$  on the day of capture (Fig. 1). Single consignments of scallops were air freighted from the Isle of Man to Belfast on 11 November 2003, 19 July 2004 and 9 December 2004, corresponding to the three sampling occasions and delivered by road to Letterkenny Institute of Technology for sample preparation and DA analysis.

### 2.2. Scallop tissue preparation

Shell dimensions (shell length, height and depth) of each scallop ( $n = 261$ ) were measured and scallops aged using ring analysis. Each scallop was dissected and total tissue weight and individual weight of hepatopancreas (HP), gonad and adductor muscle determined. Composite samples of gonad and of adductor muscle from each fishing ground were prepared for analysis.

### 2.3. Domoic acid extraction and analysis by HPLC/UV and HPLC/DAD

DA was extracted from scallop tissue samples using procedures based on Quilliam et al. (1995), with some modifications. Extraction was performed from approximately 4.0 g of tissue homogenate with 16 ml of 50:50 extraction solvent (methanol:water) in a blender (Ultra



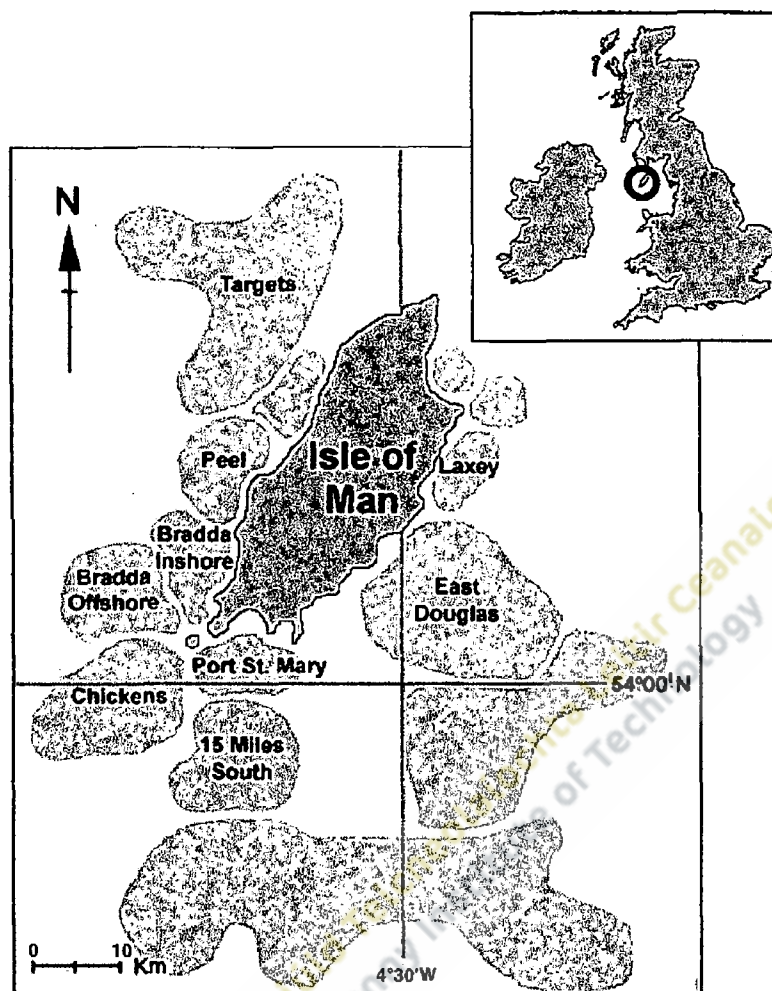


Fig. 1. Map showing location of the main king scallop-fishing grounds around the Isle of Man. Grounds sampled in this study are labelled in bold.

Turrax T25, IKA®-Works) for 4 min. DA was extracted from each hepatopancreas individually. Where hepatopancreas weight exceeded 4.0 g, it was homogenised and a 4.0 g sub-sample used for extraction. Each gonad composite and each adductor composite was sub-sampled in triplicate and DA extracted. Following extraction, homogenates were centrifuged at 3800 rpm for 30 min. Each supernatant was filtered using a methanol-compatible 0.45 µm syringe filter and the combined concentration of DA and *epi*-DA in the filtered extract determined using a Shimadzu HPLC/UV and following equipment upgrade a Shimadzu HPLC equipped with a SPD-M10A diode array detector (Mason Technology, Dublin). In those instances, where the DA concentration exceeded that of the highest standard, the filtered extract was diluted with extraction solvent and the measurement of DA and *epi*-DA repeated. All solvents used were HPLC grade (Lennox Laboratories, Dublin). Eluant consisted of 9% acetonitrile in deionised

water plus 1% TFA (trifluoroacetic acid). HPLC flow rate was 1.0 ml min<sup>-1</sup>, injection volume was 20 µl and the column used was a Vydac 10 µm C18 25 cm × 4.6 mm. The HPLC system was calibrated using six DA calibration standards between 0.2 and 10 µg ml<sup>-1</sup> prepared from Certified Reference Standards supplied by NRC, Canada. Calibration curves were prepared with each sample batch.

Statistical analysis of all data was performed using SPSS, Version 12.

### 3. Results

The mean, standard deviation and coefficient of variation (CV) of shell length, shell height, total tissue weight and weights of hepatopancreas, gonad and adductor muscle of scallop from the Isle of Man fishing grounds (Fig. 1) sampled in October 2003, June 2004 and October 2004 are provided in Table 1. Mean shell

Table 1

Physical characteristics of *Pecten maximus* from Isle of Man sampling sites

	Targets	Peel	Bradda Inshore	Bradda Offshore	Chickens	15 MS–Port St. Mary	East Douglas	Laxey
October 2003								
Shell length (mm)								
Mean	116.7	104.7	102.1	112.4	a	a	115.9	106.1
S.D.	14.9	24.9	16.5	7.4	a	a	11.3	12.2
% CV	12.8	23.8	16.2	6.6			9.8	11.5
Shell height (mm)								
Mean	103.3	93.5	92.1	101.2	a	a	105.1	95.5
S.D.	11.1	21.1	14.0	6.6	a	a	10.6	11.0
% CV	10.7	22.6	15.3	6.5			10.1	11.5
Total tissue weight (g)								
Mean	76.0	52.5	52.4	62.8	a	a	55.2	51.7
S.D.	24.4	32.7	23.3	10.5	a	a	18.0	15.4
% CV	32.0	62.4	44.5	16.7			32.6	29.9
Hepatopancreas weight (g)								
Mean	3.1	2.7	2.6	3.0	a	a	2.4	1.9
S.D.	1.0	1.8	1.2	0.7	a	a	0.9	0.6
% CV	31.4	66.0	45.4	22.8			37.2	31.3
Gonad weight (g)								
Mean	8.9	3.1	2.1	3.4	a	a	4.8	1.6
S.D.	7.4	2.8	1.9	1.5	a	a	5.8	0.9
% CV	82.9	90.9	91.5	45.4			120.3	56.3
Adductor weight (g)								
Mean	b	b	b	b	a	a	b	b
S.D.	b	b	b	b	a	a	b	b
% CV								
June 2004								
Shell length (mm)								
Mean	105.3	106.6	107.8	104.9	104.6	107.6	104.7	107.3
S.D.	3.0	3.5	2.7	3.6	4.0	4.8	3.7	2.0
% CV	2.9	3.3	2.5	3.4	3.8	4.5	3.5	1.8
Shell height (mm)								
Mean	95.6	96.3	97.8	94.7	94.3	96.5	93.9	96.2
S.D.	2.4	3.5	3.7	3.6	3.3	4.9	3.1	2.9
% CV	2.5	3.6	3.8	3.8	3.5	5.1	3.3	3.0
Total tissue weight (g)								
Mean	43.5	43.5	48.3	35.5	34.1	38.5	41.0	40.4
S.D.	2.8	4.3	7.7	2.2	3.2	6.0	3.6	2.8
% CV	6.5	9.9	16.0	6.2	9.3	15.5	8.8	6.9
Hepatopancreas weight (g)								
Mean	2.8	3.3	3.0	2.4	2.3	3.1	2.5	2.1
S.D.	0.3	0.5	0.5	0.3	0.3	0.5	0.4	0.3
% CV	10.1	15.0	16.1	12.9	12.1	16.8	16.7	13.4
Gonad weight (g)								
Mean	5.8	3.4	5.4	1.7	1.9	2.7	5.5	4.4
S.D.	1.3	1.6	3.4	0.9	0.5	0.7	1.3	1.1
% CV	23.0	46.8	63.3	50.3	25.9	26.9	22.9	24.1
Adductor weight (g)								
Mean	19.0	19.8	23.1	18.0	17.3	18.3	19.0	19.2
S.D.	1.3	1.3	4.3	1.2	1.9	3.7	1.8	2.0
% CV	6.8	6.6	18.6	6.7	11.0	20.2	9.5	10.4

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Table 1 (Continued)

	Targets	Peel	Bradda Inshore	Bradda Offshore	Chickens	15 MS-Port St. Mary	Eau Douglas	Laxey
October 2004								
Shell length (mm)								
Mean	101.0	100.8	102.5	101.7	101.9	105.8	101.8	102.0
S.D.	1.9	2.0	3.0	2.2	3.1	3.3	2.1	2.1
%CV	1.9	2.0	2.9	2.2	3.0	3.1	2.0	2.0
Shell height (mm)								
Mean	90.9	90.5	92.0	92.5	92.1	95.0	90.6	92.8
S.D.	2.2	2.5	2.9	2.6	3.6	3.7	2.8	3.6
%CV	2.4	2.8	3.1	2.8	3.9	3.9	3.1	3.8
Total tissue weight (g)								
Mean	44.9	47.3	47.7	48.3	41.7	53.8	42.5	43.9
S.D.	4.3	4.3	3.0	7.7	8.4	4.2	4.9	5.1
%CV	9.5	9.0	6.4	15.9	20.1	7.7	11.6	11.6
Hepatopancreas weight (g)								
Mean	2.7	2.6	2.7	3.0	2.2	3.0	2.2	2.0
S.D.	0.3	0.3	0.2	0.5	0.4	0.4	0.3	0.2
%CV	10.9	11.4	8.0	15.6	17.1	12.9	11.5	9.9
Gonad weight (g)								
Mean	1.6	1.2	1.7	1.5	5.1	1.4	2.4	1.8
S.D.	1.0	0.3	0.6	0.9	2.0	1.0	1.8	1.6
%CV	60.9	26.8	35.0	62.6	38.2	71.6	76.9	92.8
Adductor weight (g)								
Mean	18.5	18.5	20.7	20.9	17.1	19.9	16.4	17.9
S.D.	1.6	1.5	1.7	2.7	2.7	1.5	2.1	1.8
%CV	8.6	8.1	8.2	12.9	15.8	7.5	12.8	10.1

<sup>a</sup> Samples not provided from site.

<sup>b</sup> Adductor muscle not available for analysis.

lengths in October 2003 ranged from 102.1 to 116.7 mm with CVs ranging from 6.6% to 23.8%. By comparison scallops from June 2004 and October 2004 exhibited reduced ranges in both mean shell length and CV demonstrating that samples on these later dates were of a more uniform shell length. Mean total tissue weights and hepatopancreas weights of individual scallops showed greater variability than mean shell lengths; CVs ranging from 16.7% to 62.4% and 22.8% to 66.0%, respectively, on the first sampling occasion. Mean gonad weight showed most variability of the physical characteristics measured; CVs ranging from 45.4% to 120.3% on the first sampling occasion.

Mean DA concentrations in hepatopancreas, gonad and adductor muscle from each fishing ground on the three sampling occasions are provided in Table 2. Comparison of toxin concentrations between the different scallop tissues demonstrated that highest concentrations always occurred in hepatopancreas followed by gonad and adductor muscle. Mean concentrations of DA in hepatopancreas ranged from 296.3 to 0.2  $\mu\text{g g}^{-1}$ , below the limit of quantification; in gonad from 29.5  $\mu\text{g g}^{-1}$  to below the limit of detection and in adductor muscle from

7.3  $\mu\text{g g}^{-1}$  to below the limit of detection. The EU regulatory concentration for DA of 20  $\mu\text{g g}^{-1}$  was exceeded in 7 of 22 gonad samples but not in any adductor muscle samples.

High levels of inter-animal variability of DA concentration in hepatopancreas from each fishing ground on the three sampling occasions were recorded, CVs ranging from 16.1% to 70.0% excluding samples from sites on the east of the Isle of Man in October 2003 which contained low mean DA concentrations with several individual scallops below the limit of quantification.

Considerable spatial variation in DA concentration was recorded on each sampling occasion, best exemplified by toxin concentration in hepatopancreas, the tissue with the highest concentration. Particularly salient was the difference in mean DA concentration in hepatopancreas from fishing grounds to the west and southwest of the Isle of Man compared with those from grounds to the east of the Isle of Man. On the three sampling occasions, mean DA concentrations in hepatopancreas from sites on the west and southwest were 236.5, 63.7 and 172.7  $\mu\text{g g}^{-1}$  compared to 0.9, 6.2 and 2.8  $\mu\text{g g}^{-1}$ , respectively, from

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Table 2  
Variation in DA concentration in scallop hepatopancreas, gonad and adductor muscle in October 2003, June 2004 and October 2004 from fishing grounds around the Isle of Man

Fishing ground	Mean water depth (m)	October 2003			June 2004			October 2004		
		Concentration of DA ( $\mu\text{g g}^{-1}$ )			Concentration of DA ( $\mu\text{g g}^{-1}$ )			Concentration of DA ( $\mu\text{g g}^{-1}$ )		
		HP	Gonad	Adductor	HP	Gonad	Adductor	HP	Gonad	Adductor
Targets	28.8 $\pm$ 1.5	207.0 $\pm$ 63.6	6.4 $\pm$ 0.2	a	87.9 $\pm$ 50.0	19.0 $\pm$ 0.6	4.4 $\pm$ 0.5	130.1 $\pm$ 27.7	14.2 $\pm$ 1.3	1.4 $\pm$ 0.3
Peel	34.1 $\pm$ 0.7	250.9 $\pm$ 129.5	22.0 $\pm$ 1.4	a	111.7 $\pm$ 27.5	27.3 $\pm$ 1.3	4.8 $\pm$ 0.3	176.7 $\pm$ 41.3	27.8 $\pm$ 0.0	1.6 $\pm$ 0.3
Bradda Inshore	33.4 $\pm$ 0.6	191.7 $\pm$ 134.2	8.5 $\pm$ 1.3	a	49.4 $\pm$ 21.4	19.2 $\pm$ 0.5	6.5 $\pm$ 0.6	144.4 $\pm$ 23.2	20.4 $\pm$ 0.7	1.5 $\pm$ 0.9
Bradda Offshore	41.4 $\pm$ 1.3	296.3 $\pm$ 104.5	20.5 $\pm$ 1.1	a	39.3 $\pm$ 22.0	17.1 $\pm$ 0.2	5.9 $\pm$ 0.4	207.4 $\pm$ 67.1	26.2 $\pm$ 0.8	2.6 $\pm$ 0.2
Chickens	63.6 $\pm$ 1.2	NS	NS	NS	24.9 $\pm$ 13.4	15.3 $\pm$ 0.2	4.2 $\pm$ 0.0	110.7 $\pm$ 30.0	9.3 $\pm$ 0.9	1.6 $\pm$ 0.9
15 MS-Port St. Mary	64.0 $\pm$ 6.3	NS	NS	NS	68.8 $\pm$ 30.5	29.5 $\pm$ 0.3	7.3 $\pm$ 0.9	267.0 $\pm$ 45.6	17.8 $\pm$ 2.6	2.6 $\pm$ 0.3
East Douglas	34.7 $\pm$ 0.9	1.6 $\pm$ 1.6	0.8 $\pm$ 0.0	a	7.7 $\pm$ 3.6	6.2 $\pm$ 0.4	0.9 $\pm$ 0.0	2.4 $\pm$ 1.2	0.9 $\pm$ 0.2	ND
Laxey	26.9 $\pm$ 0.9	0.2 $\pm$ 0.5	ND	a	4.6 $\pm$ 2.6	4.8 $\pm$ 0.4	0.7 $\pm$ 0.2	3.2 $\pm$ 0.9	0.9 $\pm$ 0.4	0.9 $\pm$ 0.4

NS, samples not provided; ND, not detected.

a Adductor muscle not available for analysis.

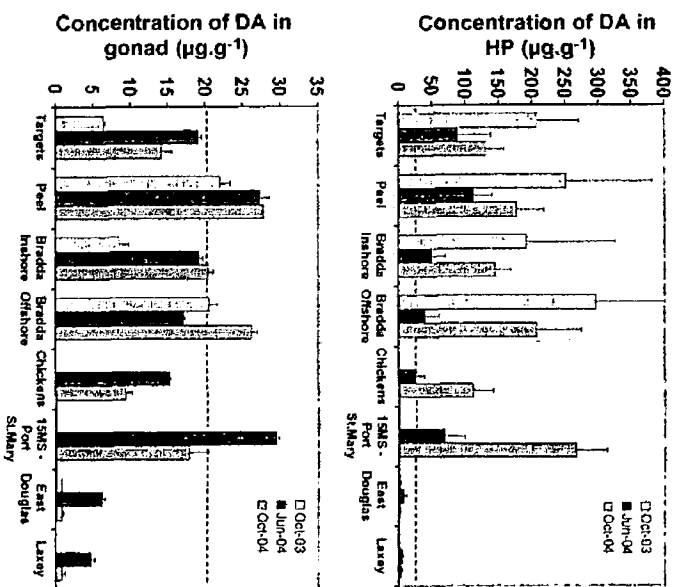


Fig. 2. Mean DA concentration in the hepatopancreas and gonad of king scallop from IOM fishing grounds on three sampling occasions (error bars = S.D.). Dashed lines in both charts represent 20  $\mu\text{g g}^{-1}$  legal limit.

sites on the east of the Isle of Man. Comparison of all results of mean DA concentration in hepatopancreas from the west and southwest (six fishing grounds) with those from the east (two fishing grounds) confirmed the significant difference observed ( $t = 10.649$ ,  $p < 0.001$ ). Over the study duration DA concentration in an individual hepatopancreas or composite tissue sample of gonad or adductor never exceeded the regulatory level of 20  $\mu\text{g g}^{-1}$  in scallops from the east of the Isle of Man.

Significant differences in DA concentration in hepatopancreas between October 2004 and June 2004 ( $t = -7.676$ , d.f. = 190,  $p < 0.001$ ) and between October 2004 and October 2003 ( $t = 9.458$ , d.f. = 152,  $p < 0.001$ ) were recorded. Higher DA concentrations in hepatopancreas were recorded in the two winter samples compared to the summer sample. Since DA concentration is influenced by both the mass of DA and the weight of the organ within which it is present, both concentration of DA (Table 2, Fig. 2) and mass of DA (Table 3, Fig. 3) in hepatopancreas and gonad were considered. Similar trends in the concentration and mass of DA in hepatopancreas were exhibited both between different fishing grounds and seasonally. By contrast, in gonad tissue, trends between the concentration of toxin and mass of toxin were not consistent between fishing grounds or seasonally.

Table 3

Variation in mass of DA in scallop hepatopancreas and gonad in October 2003, June 2004 and October 2004 from fishing grounds around the Isle of Man

Fishing ground	October 2003		June 2004		October 2004	
	Mass of DA ( $\mu\text{g}$ )		Mass of DA ( $\mu\text{g}$ )		Mass of DA ( $\mu\text{g}$ )	
	HP	Gonad	HP	Gonad	HP	Gonad
Targets	624.3 $\pm$ 223.6	56.3 $\pm$ 46.7	241.6 $\pm$ 134.9	110.3 $\pm$ 25.4	358.5 $\pm$ 96.7	23.3 $\pm$ 14.2
Peel	812.6 $\pm$ 807.1	68.7 $\pm$ 62.5	370.3 $\pm$ 102.6	93.6 $\pm$ 43.8	464.7 $\pm$ 125.0	32.8 $\pm$ 8.8
Bradda Inshore	604.6 $\pm$ 559.6	17.9 $\pm$ 16.4	148.1 $\pm$ 58.9	104.3 $\pm$ 66.0	384.3 $\pm$ 46.4	34.4 $\pm$ 12.1
Bradda Offshore	925.2 $\pm$ 509.4	69.6 $\pm$ 31.6	93.6 $\pm$ 45.8	29.9 $\pm$ 15.0	634.1 $\pm$ 284.1	38.4 $\pm$ 24.0
Chickens	NS	NS	55.3 $\pm$ 29.5	29.6 $\pm$ 7.7	238.9 $\pm$ 72.4	47.7 $\pm$ 18.2
15 MS-Port St. Mary	NS	NS	216.0 $\pm$ 99.1	79.8 $\pm$ 21.5	792.2 $\pm$ 131.0	24.6 $\pm$ 17.6
East Douglas	3.7 $\pm$ 3.3	4.1 $\pm$ 4.9	18.4 $\pm$ 6.8	34.5 $\pm$ 7.9	5.5 $\pm$ 3.1	2.2 $\pm$ 1.7
Laxey	2.3 $\pm$ 0.9	ND	9.9 $\pm$ 5.6	21.4 $\pm$ 5.1	6.5 $\pm$ 2.0	2.0 $\pm$ 1.8

NS, samples not provided; ND, not detected.

Relationships between DA concentrations in hepatopancreas and physical characteristics of scallops were investigated in each of the sampled fishing grounds on each sample date (Table 4). In October 2003 the concentration of DA in hepatopancreas exhibited a significant relationship ( $p < 0.05$ ) with scallop shell length in Peel ( $R^2 = 0.48$ ,  $n = 12$ ), Bradda Inshore ( $R^2 = 0.68$ ,  $n = 11$ ) and Bradda Offshore ( $R^2 = 0.54$ ,  $n = 12$ ); with total tissue weight in Peel ( $R^2 = 0.55$ ,  $n = 12$ ), Bradda Inshore ( $R^2 = 0.67$ ,  $n = 11$ ) and Bradda Offshore ( $R^2 = 0.54$ ,

$n = 12$ ); and with hepatopancreas weight in Peel ( $R^2 = 0.49$ ,  $n = 12$ ), Bradda Inshore ( $R^2 = 0.65$ ,  $n = 11$ ) and Bradda Offshore ( $R^2 = 0.49$ ,  $n = 12$ ). In the other sites sampled in October 2003 no relationships ( $p > 0.05$ ) were exhibited between DA concentration in hepatopancreas and these physical characteristics. Scallop samples exhibiting significant relationships in October 2003 had shell lengths ranging from 75 to 140 mm in Peel, 78 to 125 mm in Bradda Inshore and 102 to 126 mm in Bradda Offshore. On the two other sampling dates, significant relationships between DA concentration in hepatopancreas and scallop shell length ( $R^2 = 0.53$ ,  $n = 12$ ), but not total tissue weight ( $R^2 = 0.15$ ,  $n = 12$ ) or hepatopancreas weight ( $R^2 < 0.01$ ,  $n = 12$ ) were exhibited by scallops from Chickens in October 2004, however scallops in this sample ranged from only 100 to 110 mm shell length. DA concentration in hepatopancreas from the Laxey sample in June 2004 exhibited significant relationships with scallop shell length ( $R^2 = 0.44$ ,  $n = 12$ ) and total tissue weight ( $R^2 = 0.42$ ,  $n = 12$ ) but not hepatopancreas weight ( $R^2 = 0.00$ ,  $n = 12$ ). Shell length and toxin concentration in this Laxey sample ranged from 102 to 110 mm and 1.9 to 9.3  $\mu\text{g g}^{-1}$ , respectively.

Relationships between DA concentrations in hepatopancreas and water depth on each sample date were determined. Water depth ranged between  $26.9 \pm 0.9$  m and  $64.0 \pm 6.3$  m at the sampling sites in the different fishing grounds. There was no relationship between mean DA concentration in hepatopancreas of scallops from Isle of Man fishing grounds and mean water depth in October 2003 ( $R^2 = 0.30$ ,  $n = 6$ ), June 2004 ( $R^2 < 0.01$ ,  $n = 8$ ) or October 2004 ( $R^2 = 0.26$ ,  $n = 8$ ). Given the significant difference in toxin concentration between fishing grounds off the west and southwest of the Isle of Man and those on the east of the Isle of Man,

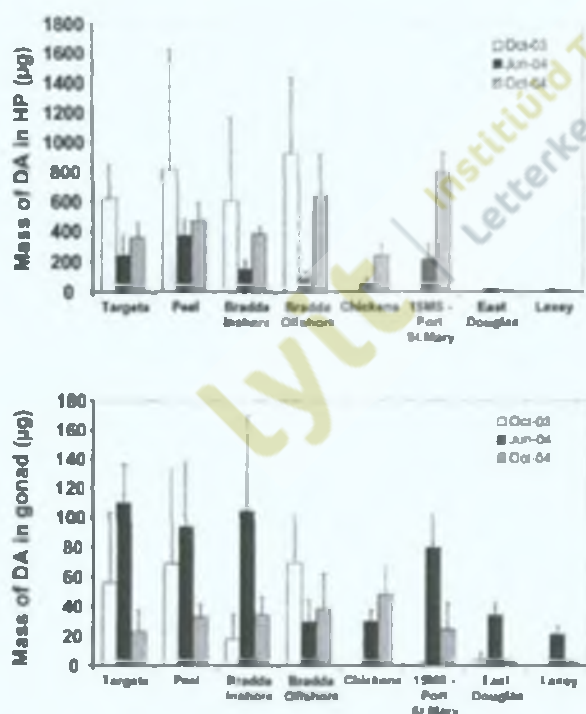


Fig. 3. Mean DA mass in the hepatopancreas and gonad of king scallop from IOM fishing grounds on three sampling occasions (error bars = S.D.).

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Table 4  
Relationship between DA in scallop hepatopancreas and shell length in October 2003, June 2004 and October 2004 from fishing grounds around the Isle of Man

Fishing ground	October 2003				June 2004				October 2004			
	<i>n</i>	Shell length range (mm)	Concentration of DA in HP ( $\mu\text{g g}^{-1}$ )	$R^2$	<i>n</i>	Shell length range (mm)	Concentration of DA in HP ( $\mu\text{g g}^{-1}$ )	$R^2$	<i>n</i>	Shell length range (mm)	Concentration of DA in HP ( $\mu\text{g g}^{-1}$ )	$R^2$
Targets	12	100–144	107.6–325.5	0.20	12	100–110	44.2–223.2	0.16	12	99–105	75.4–166.0	0.08
Peel	12	75–140	124.1–538.6	0.48	12	100–110	65.8–151.1	0.02	12	87–95	124.8–251.0	0.30
Bradda Inshore	11	78–125	33.4–404.0	0.68	12	102–110	14.2–77.2	0.07	12	100–109	109.9–179.9	0.00
Bradda Offshore	12	102–126	156.3–498.9	0.54	12	100–110	8.1–82.7	0.00	12	99–105	114.7–334.8	0.38
Chickens		NS	NS		12	101–115	7.4–46.0	0.08	12	100–110	68.7–167.5	0.53
15 MS-Port St. Mary		NS	NS		12	102–120	14.8–123.2	0.08	12	100–110	173.3–354.2	0.07
East Douglas	10	102–142	0.0–4.8	0.10	12	100–110	3.1–14.9	0.22	12	99–105	1.2–5.1	0.00
Laxey	12	85–119	0.0–1.5	0.07	12	102–110	1.9–9.3	0.44	12	100–105	1.9–5.0	0.28

NS, samples not provided.

this dataset was re-evaluated with exclusion of the two low concentration sites from the east coast. Mean DA concentration in hepatopancreas of scallops from west and southwest fishing grounds exhibited no relationship ( $p > 0.05$ ) with mean water depth in June 2004 ( $R^2 = 0.26$ ,  $n = 6$ ) or October 2004 ( $R^2 = 0.11$ ,  $n = 6$ ) however there was a significant relationship in October 2003 ( $R^2 = 0.62$ ,  $n = 4$ ) though the water depth ranged from only 28.8 to 41.4 m, the two deeper water sites in the southwest not being sampled on this date.

#### 4. Discussion

Accumulation of DA by king scallop and anatomical distributions of the toxin similar to that recorded in this study have been widely reported (Arévalo et al., 1998; Campbell et al., 2001; Gallacher et al., 2001; Blanco et al., 2002; James et al., 2005; Bogan et al., 2006a). The highest concentration of DA in hepatopancreas of an individual king scallop over the study duration was  $558.6 \mu\text{g g}^{-1}$ , considerably below the highest concentrations of  $1348.1 \mu\text{g g}^{-1}$  reported by Bogan et al. (2006a) for scallops from waters off the southeast of Ireland,  $2083 \mu\text{g g}^{-1}$  reported by Arévalo et al. (1998) for scallops from northwest Spain and  $2820 \mu\text{g g}^{-1}$  reported by James et al. (2005) for scallops from the west of Ireland. Toxin concentrations in gonad were much lower than in hepatopancreas; the highest concentration recorded in a gonad composite of  $29.9 \mu\text{g g}^{-1}$  exceeding the maximum of  $9.59 \mu\text{g g}^{-1}$  recorded by Bogan et al. (2006b) in scallops from Clew Bay, Ireland but being below the values of  $69.9 \mu\text{g g}^{-1}$  reported by Arévalo et al. (1998) and  $75.47 \mu\text{g g}^{-1}$  reported by Campbell et al. (2001).

Inter-animal variability in DA concentration in hepatopancreas, CVs ranging from 16.1% to 70.0% in Isle of Man fishing grounds were high by comparison to the CVs of 28% to 39% in four sites off the southeast coast of Ireland (Bogan et al., 2006a). Similar high inter-animal variability in DA concentration in other king scallop tissues have been reported (Arévalo et al., 1998; Campbell et al., 2001; FSA, 2001).

Scallops from fishing grounds within relatively close proximity to each other exhibited significant spatial variation in DA concentration in hepatopancreas. For example in October 2004, mean DA concentration in hepatopancreas from the Chickens ground was  $110.7 \mu\text{g g}^{-1}$ , but  $267.0 \mu\text{g g}^{-1}$  in hepatopancreas from the adjacent Port St. Mary–15 Miles South ground. Similar high levels of spatial variation in DA concentration have been reported in king scallops (Campbell et al., 2001; FSA, 2001; Bogan et al., 2006a) and razor clams (Wekell



et al., 2002). Spatial variability in DA concentration in whole scallops on this scale presents difficulties for regulatory authorities with responsibility for area management of the status of scallop harvesting—viz. should an area be open or closed for scallop fishing, in particular where both fishing grounds as in this case are located within one area for management purposes.

The most significant difference in DA concentration between the eight sampling sites was that reported between fishing grounds on the west and southwest of the Isle of Man compared to those on the east of the Isle of Man. Over the study duration mean DA concentration in composite gonad or composite adductor muscle samples from the east coast never exceeded  $20 \mu\text{g g}^{-1}$ , indeed the highest DA concentration recorded in an individual hepatopancreas from an east coast site (East Douglas or Laxey) was  $14.9 \mu\text{g g}^{-1}$ . A similar spatial trend identifying “problem” grounds for DA in Manx territorial waters as those to the west and to a lesser extent the southwest of the Isle of Man was reported by Shammon et al. (2004). On only one occasion between September 2002 and November 2005 has DA concentration in whole scallop exceeded  $20 \mu\text{g g}^{-1}$  in king scallop from an east coast site (Shammon et al., 2004; DLGE, 2005). Given the prolonged retention of this toxin and exceedingly slow rate of depuration in this species, the slowest of any of the scallop species so far investigated (Blanco et al., 2002), the low DA concentrations in hepatopancreas from east coast sites suggested that scallops from these sites were not exposed to toxic *P. nitzschia* cells to the same extent as scallops from west and southwest sites. Such differences suggest that oceanographic conditions on either side of the Isle of Man differ significantly and that little mixing between the two water masses occurs. Waters in the eastern Irish Sea are generally less than 50 m deep, there are extensive shallow coastal areas and long residence times for the water mass have been reported (Gowen and Stewart, 2005; Kennington et al., 2005). Waters in the western Irish Sea have a shorter residence time and are generally deeper; a trough 80–100 m deep extending north south, through which an appreciable part of the main south to north water flow passes (Ramster and Hill, 1969; Slinn, 1974; Gowen and Stewart, 2005). Deep waters and weak tidal currents ( $<25 \text{ cm s}^{-1}$ ) south west of the Isle of Man allow the seasonal development of stratification, which can become very stable except in the area having a near-surface gyre driven by density gradients associated with cold bottom water (Slinn, 1974; Hill et al., 1994; Gowen and Stewart, 2005; Kennington et al., 2005). In moderately stratified and warm waters in the Gulf of Mexico, increased abundance of *P. nitzschia*

cells has been recorded (Dortch et al., 1997). Likewise increased DA production was associated with coastal upwelling off the coast of California (Trainer et al., 2000). Hence to the west of the Isle of Man, it is possible that stratification combined with increased sea surface temperatures recorded over the last 10 years (Shammon et al., 2004) or the near-surface gyre to the south west of the Isle of Man may be responsible for increased production of toxic *P. nitzschia* cells compared to levels in waters to the east of the Isle of Man. In addition several frontal systems have been identified to the north and south of these stratified regions in the western Irish Sea (Kennington et al., 2005) and such fronts might also be a contributory factor in the development of *P. nitzschia* blooms, as noted in waters off the southeast of Ireland (Cusack, 2002). The salinity profiles in the Irish Sea suggest that limited water exchange between the eastern and western water masses occurs, although radionuclide distributions indicate some east west transport, probably north of the Isle of Man (Leonard et al., 1997; Gowen and Stewart, 2005). Elevated *P. nitzschia* cell counts have been reported in waters in the western Irish Sea during summer months although to date it has not been possible to establish a link between the spatial distribution of grouped *P. nitzschia* species and DA concentrations in scallop tissues (Shammon et al., 2004).

Significant differences in concentration of DA in hepatopancreas between the three sampling occasions were reported, the two winter samples (October 2003 and October 2004) exhibiting higher concentrations than the summer sample (June 2004). Scallops from the west and southwest Isle of Man fishing grounds monitored between September 2002 and December 2003 attained their highest concentration of  $120 \mu\text{g g}^{-1}$  DA in whole scallops during the winter of 2002 (Shammon et al., 2004). Highest concentrations of DA during the winter months of 1999/2000 were also reported in king scallop from the west of Ireland (James et al., 2005) despite the absence of high *P. nitzschia* cell counts. Temporal studies in Clew Bay, west of Ireland from February 2003 to February 2004 reported highest concentrations of DA in hepatopancreas from February 2003 to April 2003 (Bogan et al., 2006b, 2006c). However, based on the results of this short-term study there are insufficient sampling dates and the sampling interval is too large to suggest that peak DA concentrations occur during winter in king scallops from the Isle of Man.

Given that temporal and spatial changes in the size of hepatopancreas or gonad could influence the concentration of DA recorded in these tissues, both concentration and mass of toxin were determined for each sample.

The similar trends in concentration and mass of toxin in hepatopancreas, both between fishing grounds and seasonally, were attributed to the similar size of this organ in all samples, though higher variability was recorded in the first sample in October 2003. The data suggested that the concentration of DA measured in the hepatopancreas is influenced to a greater extent by the mass of DA present rather than by the size of the hepatopancreas. By contrast trends in the concentration and mass of toxin in gonad, both between fishing grounds and seasonally, were not consistent. Temporal and spatial differences between gonad DA concentration and DA mass were attributed to the lower mass of toxin present compared to hepatopancreas and the increased variation in gonad weight both seasonally, as part of the reproductive cycle, and between different fishing grounds. For example in June 2004, Bradda Inshore scallops of mean shell length  $107.8 \pm 2.7$  mm had a mean gonad weight of  $5.4 \pm 3.4$  g compared to Bradda Offshore scallops of similar mean shell length ( $104.9 \pm 3.6$  mm) with a mean gonad weight of  $1.7 \pm 0.9$  g. Trends in the mass of DA in gonad did not reflect trends in the mass of DA in hepatopancreas in contrast to the results from a temporal study in Clew Bay between February 2003 to February 2004 (Bogan et al., 2006c). Similar difficulties in understanding trends based on DA concentration in gonad have been reported elsewhere (FSA, 2001; Bogan et al., 2006c).

No relationship between DA concentration and scallop shell length has been reported from studies based on a single sampling occasion (Arévalo et al., 1998; Campbell et al., 2001). By contrast, a more detailed examination on 10 sampling occasions using a wider range of scallop shell lengths, as suggested by Campbell et al. (2001), demonstrated that DA concentration in hepatopancreas could be influenced by scallop shell length (Bogan et al., 2006b). DA concentration in hepatopancreas in October 2003 exhibited significant positive relationships with shell length, total tissue weight and hepatopancreas weight of scallops from Peel, Bradda Inshore and Bradda Offshore, but not in Targets, the other west coast fishing ground sampled on that date. In those sites in which DA concentration in hepatopancreas exhibited a positive relationship, correlation coefficients were highest with total tissue weight in contrast to the highest correlation coefficients with shell length reported by Bogan et al. (2006b). The relationship between DA concentration and scallop shell length was re-examined using shell lengths  $\geq 100$  mm, since EU Commission Decision 2002/226 refers to the testing of harvestable product, i.e. above 100 mm shell length. Similar re-examination of scallops  $\geq 100$  mm by Bogan

et al. (2006b) resulted in the absence of any correlation between DA concentration in hepatopancreas and shell length. In this study the correlation in the Peel ground was absent, that in Bradda Inshore was reduced slightly from  $R^2 = 0.68$  to 0.61 and that in Bradda Offshore remained the same since all scallops were greater than 100 mm shell length. A positive correlation between DA concentration in hepatopancreas and scallop shell length could be due to either faster toxin depuration in smaller scallops, as suggested by Bogan et al. (2006b), or alternatively faster toxin accumulation in larger scallops. Given the large interval between sample dates in this study no inference as to whether scallops were in toxin depuration or toxin accumulation mode was possible. No correlation was exhibited between DA concentration in hepatopancreas and scallop shell length on the other two sample dates with the exception of scallops from Chickens in October 2004 and Laxey in June 2004. In these samples, the range in shell length or the range in shell length and toxin concentration was considered too limited for an investigation of the relationship between these parameters.

DA concentration in hepatopancreas exhibited no correlation with water depth on any of the sampling occasions in this Isle of Man study. When sampling sites to the west of the Isle of Man were considered, samples from October 2003 exhibited a positive correlation between DA concentration in hepatopancreas and water depth, however depth ranged from only 28.8 to 41.4 m and included samples from only four of the six west coast fishing grounds under investigation. These results contrast with the negative correlations between water depth and DA concentrations in whole scallop, and scallop tissues reported elsewhere (FSA, 2001; Bogan et al., 2006a).

This investigation of DA concentrations in king scallop from around the Isle of Man contributes to our understanding of spatial variation in an area heavily dependent on the scallop fishery. The long-standing fishery for Manx scallop with continual exploitation since 1937 has resulted in a fishery now largely dependent on the strength of each recruiting year-class (Beukers-Stewart et al., 2003). Despite the many fishery management restrictions, the sustainability of the fishery remains a prime concern should poor recruitment success occur. Further investigation of the low DA concentrations on the east coast is required as an opportunity may exist for the development of a niche market for fresh in-shell scallops, a market typified by significantly higher prices than those for processed scallops and one which in many European waters cannot be supplied due to biotoxin regulations. Such an opportunity might allow increased



revenue into the fishery, particularly important if extensive closures due to biotoxins were to affect the grounds to the west of the Isle of Man or if poor recruitment were to affect the area in general.

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